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THE CLINICAL
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ANTIBIOTICS

VOLUME IV
ERYTHROMYCIN
AND OTHER
ANTIBIOTICS

THE
CLINICAL APPLICATION
OF
ANTIBIOTICS

BY
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VOLUME IV
ERYTHROMYCIN AND
OTHER ANTIBIOTICS

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PREFACE

VOLUME IV of *The Clinical Application of Antibiotics* includes discussion of such antibiotics as have not had so extensive a clinical trial as penicillin, streptomycin, chloramphenicol, or the tetracyclines. In the final chapter more recently published information regarding these latter drugs is included to help in the choice of antibiotic. Those applicable to the treatment of tuberculosis, however, are reserved for Volume II.

As in Volume III, the references given in the bibliography are not fully comprehensive, but an attempt has been made to include enough to give a fair impression of the clinical capabilities of each antibiotic or combination of antibiotics discussed. It is hoped also that there are enough to enable any reader who wishes to do so to study each subject in greater detail than in the necessarily summary account given here. It has been the author's aim not to allow personal experience to bias the discussion of each subject, and the conclusions drawn are those which appeared justified in the light of all the dependable evidence that was available.

So far as available information allowed, discussion of each antibiotic has followed the same scheme as in previous volumes: general considerations, including those properties of clinical significance; antibacterial effects; evidence of toxicity; administration; and the results of clinical trials. Further, the reader is reminded that the terms used are those employed by the authors quoted in their references. Standard abbreviations are assumed to be used interchangeably. Thus *Ps. pyocyanea*, *Bacillus pyocyaneus*, or *Ps. aeruginosa* signify the same species of organism. Likewise *Bact. friedlanderii* is equivalent to *Klebsiella* or *Klebs. pneumoniae*.

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M. E. FLOREY

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February 1959

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| <i>Antibiotics and Chemotherapy</i> | (Figs. 11 and 12) |
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| <i>New England Journal of Medicine</i> | (Figs. 2 and 10) |
| <i>Antibiotics Annual</i> | (Figs. 3 and 4) |

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CHAPTER 1

ERYTHROMYCIN

GENERAL CONSIDERATIONS

Properties of clinical importance

THIS antibiotic, first described as *Hotycin*, was isolated in the Lilly Research Laboratories by McGuire, Bunch, Anderson, Boaz, Flynn, Powell, and Smith (1952). It was produced from a sample of soil collected in the Philippine Archipelago. This sample contained a streptomycete which was named 'erythreus' by Waksman and Henrici. The active principle in the mould was isolated and worked up by a co-operative effort. It was found, when crystalline, to be a basic compound slightly soluble in water (2 mg per ml) and very much more so in organic solvents. It was active against both Gram-negative and Gram-positive bacteria, and also certain mycobacteria such as the H37 Rv strain of *Mycobacterium tuberculosis*. Tests *in vivo* demonstrated that the viruses of lymphogranuloma venereum and mouse meningo-pneumonitis were inhibited in egg embryos, as were also certain rickettsiae. *E. histolytica*, *Sp. novyi*, and *Trichomonas vaginalis* responded to the action of erythromycin both *in vitro* and *in vivo*. The susceptibility of these 2 species to this antibiotic *in vivo* was later confirmed by McCowen, Callender, Lawlis, and Brandt (1953). Oxyurids and toxoplasma were also found to be susceptible.

The toxicity of the drug was found to be low in mice. A dose of 2 G per kg of weight was tolerated when given by mouth and 1 G per kg when given subcutaneously. The LD₅₀ was 1.8 G per kg. Dogs also tolerated a fairly large dose (100 mg per kg) and both dogs and mice could continue daily treatment for 2 weeks without suffering any apparent ill effect. When given by mouth to dogs the antibiotic was readily detected in the blood, urine, faeces, and cerebrospinal fluid, the highest level in the blood being about 1 hour after ingestion of the dose, detectable concentrations were still present at the end of approximately 8 hours.

These early investigations were elaborated by Haight and Finland (1952 a and b). They found that the stability of sterile solutions of the antibiotic was little impaired after 8 weeks at 4° C or room temperature ($\pm 25^{\circ}$ C). At 37° C the activity in solution began to decline from the 4th day onwards. Heating above 80° C for 15 minutes or more caused some loss of activity, and further activity was lost when the temperature was raised to 100° C. The activity of erythromycin was found to be greatest in an alkaline medium (pH 8.5) and a slight amount was lost after filtration through a Berkefeld filter. The activity of the antibiotic against the organisms investigated was independent of the inoculum size, and it was not affected by many substances commonly used in laboratories or found in the body, such as sodium chloride, glucose, sodium thioglycollate, cysteine hydrochloride, semicarbazide, urea, glutamic acid, para-aminobenzoic acid, folic acid, penicillinase, or serum. Moreover, no inhibiting substance was found in Gram-negative bacilli which were resistant to the antibiotic.

Erythromycin was found to be most active against rapidly multiplying organisms. The rate of death of the organisms was proportional to the concentration of the antibiotic, and the time required to kill an inoculum was proportional to the size of the inoculum.

Antibacterial activity

Haight and Finland (1952 *e*) found that pneumococci and haemolytic streptococci were the micro organisms most sensitive to this antibiotic, and that coliform bacilli, *B. proteus*, and *Ps. pyocyanea* were completely resistant.

Table 1, compiled from the studies of several investigators, gives a list of pathogens and their susceptibility to erythromycin according to two different methods of testing. It will be seen from the table that, where both broth dilution tests and tests on agar were carried out, the broth dilution test usually gave a higher figure for the lowest inhibitory concentration than that given by tests on solid media. Nevertheless many more strains were tested by the latter method and they afford some idea of the relative limits of the susceptibility to erythromycin of the micro organisms tested.

Haight and Finland concluded that weight for weight the activity of this antibiotic and that of penicillin were similar against susceptible organisms, its activity *in vitro* against Gram positive organisms is thus greater than that of the tetracyclines or chloramphenicol.

Bactericidal concentrations

As is usual in antibiotics which are found to be bactericidal, the concentrations as well as the time required for killing the organisms were found to be higher than those necessary for inhibition of growth *in vitro*. Heilman, Herrell, Wellman, and Geraci (1952) found that 5 to 10 μg per ml were usually required to kill staphylococci as compared with an upper limit of only 2.0 μg per ml for inhibition of growth. The drug required 24 hours at a concentration of 1 μg per ml to kill a strain of *Str. mitis* which was inhibited by only 0.1 μg per ml. Little bactericidal effect was noted against *Br. suis*. Thus it would seem that, for maximum effect, considerably higher concentrations should be aimed at *in vivo* than those needed simply to inhibit the pathogens concerned.

Resistance—acquired and cross resistance

Resistance to the drug was developed rapidly in enterococci and *Staph. aureus* (Haight and Finland, 1952 *c*; Heilman *et al.*, 1952; Schneerson, 1955). Resistance could also be induced in the pneumococcus and more slowly in group A haemolytic streptococcus and *Str. viridans*. When these strains became resistant to erythromycin they none the less retained their original degree of susceptibility to penicillin, streptomycin, chlortetracycline, oxytetracycline, chloramphenicol, bacitracin, polymyxin, and neomycin, except for 1 strain of type III pneumococcus in which the sensitivity to these antibiotics had actually increased. On the other hand, 30 strains of *Str. faecalis*, which were first exposed to and inhibited by erythromycin, were not found by Heilman *et al.* (1952) necessarily to be inhibited by other antibiotics. In fact 16 of these strains were resistant to chlortetracycline, oxytetracycline, chloramphenicol, and streptomycin. Similarly 30 strains of *Staph. aureus*,

TABLE 1 INHIBITION OF REPRESENTATIVE STRAINS OF BACTERIA
BY ERYTHROMYCIN (IN μG PER ML)

| Micro organisms | By broth dilution test | By agar dilution test |
|--|------------------------|-----------------------|
| <i>Actinomyces israeli</i> | 0.1-0.6 | |
| <i>Aerobacter aerogenes</i> | 100 | 50-200 or more |
| <i>Bacillus anthracis</i> | | 0.6 |
| <i>Br. abortus</i> | | 10.0 |
| <i>Br. melitensis</i> | 1.56 | 0.3 |
| <i>Br. suis</i> | 1.56 | 0.3-0.6 |
| <i>Candida albicans</i> A 17 | | >50 |
| <i>Cl. perfringens</i> | 1.0-5.0 | |
| Other clostridia | 0.09-2.0 | |
| <i>C. diphtheriae</i> | 0.001-0.02 | 0.003-3.12 |
| Other corynebacteria | | 0.003-0.08 |
| <i>E. coli</i> | 25->100 | 50-200 |
| <i>Ery. rhusiopathiae</i> | | 0.08-0.2 |
| <i>Haemophilus influenzae</i> | 1.25-1.56 | 0.4-2.0 |
| <i>Haemophilus pertussis</i> | 0.31 | 0.2 |
| <i>Klebs. pneumoniae</i> | >100 | 6.2->200 |
| <i>Leptospira icterohaemorrhagiae</i> * | | |
| <i>Listeria monocytogenes</i> | 0.156-0.3 | |
| Mycobacteria | 20-40 | 1.6† >6.2 |
| <i>Neisseria catarrhalis</i> \ 22 | | 1.56 |
| <i>Neisseria gonorrhoeae</i> | 0.039 | 0.04-1.0 |
| <i>Neisseria meningitidis</i> | 0.313-5.0 | 0.10-1.56 |
| <i>Nocardia asteroides</i> | 60-100 | |
| <i>Pasteurella</i> | 0.007-12.5 | |
| <i>Proteus</i> species | 5->100 | >50->200 |
| <i>Pseudomonas</i> | >5 | >50->200 |
| <i>Salmonella typhi</i> | 25->100 | 100->200 |
| Other salmonellae | 12.5->100 | >50->200 |
| <i>Shigellae</i> | 25->100 | 50->200 |
| <i>Staph. aureus</i> | 0.05-0.4 | 0.01-2.0† |
| Streptococci—haemolytic Groups A, G, C-203 | 0.01-0.1 | 0.01-3.12‡ |
| <i>Str. viridans</i> | 0.007-0.0195 | |
| <i>Str. faecalis</i> | 0.05-0.16 | 0.02-3.12 |
| <i>Str. mitis</i> | 0.156-4.0 | 0.2-3.12 |
| <i>Str. pneumoniae</i> | 0.018 | 0.02-3.12 |
| <i>Tricophyton</i> | 0.0097-0.03 | 0.009-0.09 |
| <i>Vibrio cholerae</i> | | >100 |
| <i>Vibrio comma</i> | 0.63 | |
| Yeasts and fungi | 3.13-6.25 | |
| | >50 | |

From Fusillo, Noyes, Pulaski and Tom (1953), Gorzynski and Neter (1953 a and b), Garrod and Waterworth (1956), Haight and Finland (1952 a), Heidman, Herrell, Wellman and Geraci (1952), Johnston, Solomon and Vogel (1955), McGuire *et al.* (1952), Powell, Bonece, Pittenger, Stone and Culbertson (1953), Jones and Finland (1957 a, b and c), Jones, Feldman and Finland (1957), Rantz and Rantz (1956).

* More sensitive in vivo than to benzylpenicillin and other antibiotics tested (Ormsbee 1953).

† *Mycobacterium avium* only (McGuire *et al.* (1953)).

‡ Including penicillin and streptomycin resistant strains.

§ A few haemolytic streptococci reacting with Lancefield Group D antisera only inhibited by 1.56 to more than 400 μg per ml (Jones and Finland 1957 b).

25 of which were resistant to penicillin, oxytetracycline, chlortetracycline, chloramphenicol, or streptomycin, were all found to be equally sensitive to erythromycin. When testing 288 strains of *Staph aureus* isolated from clinical material for their sensitivity to various antibiotics by the blotting paper disk method (containing 40 µg per ml when erythromycin was tested), Thomson, Rountree, and Freeman (1956) found those that were sensitive to erythromycin were unlikely to be so to other antibiotics in common use. Their results were as follows:

| Phage type | No of strains | | | |
|--------------------|------------------------|----------------------|---------------------------------------|--|
| | Erythromycin sensitive | Penicillin resistant | Penicillin and streptomycin resistant | Penicillin, streptomycin, and tetracycline resistant |
| I | 23 | 6 | | |
| II | 19 | 2 | | |
| III | 23 | 55 | 19 | 28 |
| Unclassifiable | 11 | 8 | 2 | |
| Unable to be typed | 8 | 4 | | |
| Totals | 208 | 75 | 21 | 28 |

However, except in an occasional case of a *Staph aureus* or of a non haemolytic streptococcus, cross resistance between carbomycin (p 37) and erythromycin existed (Fusillo *et al*, 1953; Hobson, 1954; Kutscher, Seguin, Lewis, Piro, Zegarelli, Rankow, and Segall, 1954) but was found by Hsie, Kotz, and Nusser (1956) not always to be complete or consistent. The finding that resistance to the drug developed readily was a warning that could not be taken too seriously for the clinical prospects of the antibiotic. Haight and Finland (1952c) found that in 2 patients with staphylococcal endocarditis the resistance to erythromycin of the organisms isolated from blood culture markedly increased in 7 to 10 days of therapy, and Hedman *et al* (1952) also detected a marked increase in resistance in a similar infection with *Str mitis*, in this case treatment with erythromycin was ineffective. Some indication that certain strains of bacteria were becoming more resistant to erythromycin as a result of its use was given in 1954 by Tunevall and Hedenius (1954). Although the method of assay used by these authors was somewhat different from those mentioned in Table 1, the upper limits of the inhibitory concentrations were often higher than those obtained by the earliest workers. Some bacteria normally considered sensitive to erythromycin were only inhibited by concentrations such as the following:

| Bacterium | No of strains tested | Upper limit of inhibitory concentration in µg per ml |
|---|----------------------|--|
| | | |
| Pneumococci | 15 | 1 |
| α streptococci | 28 | 25 |
| β " | 19 | 1 |
| γ " | 7 | 5 |
| <i>Str faecalis</i> | 61 | 125 |
| <i>Staph pyogenes</i> and other staphylococci | 212 | 5 |
| <i>C diphtheriae</i> | 6 | 1 |

Nevertheless these workers did not find any clinical evidence of resistance in the treatment of pneumonia and bronchitis when amounts of 0.8 to 1.2 G

daily, by no means a high dose, were given. Pneumococci and haemolytic streptococci were eliminated from the sputum. However, when erythromycin resistant strains of staphylococci were becoming more prevalent in patients in a hospital where the antibiotic was used freely (Lepper, Moulton, Dowling, Jackson, and Kofman, 1953 c), it was found difficult to determine whether the increased resistance of the organisms found in lesions was actually induced by the drug or whether it was due to cross infection.

Administration

Administration by mouth

The inhibitory concentrations mentioned above were obtained *in vitro*, it remained to be seen whether adequate concentrations could readily be obtained *in vivo*. Oral administration was chosen as the most convenient method. It must however be remembered that, unlike penicillin the activity of erythromycin was greater at pH 8.5 than at the lower pH found in the gastric juice and blood. For their preliminary trials Haight and Finland (1952 a) chose doses of about 250 mg initially followed by 100 to 200 mg 3 to 4 hourly. They found only a rough correlation between the size of the dose administered and the maximum concentrations obtained in the blood serum. Heilman *et al* (1952) produced estimates of the concentrations in the blood serum after different doses of the antibiotic. Though variable these did not vary more than those obtained from intramuscular injection of known amounts of penicillin. The results obtained were as follows.

| Dose | No of patients tested | μg per ml in blood at hours after last dose | | | | | |
|----------------|-----------------------|--|--------|-----|---------|---------|------------------|
| | | 1 | 3 | 4 | 6 | 9 | 12 |
| 0.3 G (1) | 1 | | 0.125 | | 0.125 | | |
| 0.5 G (1) | 3 | | | 1.4 | 1.4 | | |
| 0.3 G repeated | 4 | 0.125 | 0.25-8 | | 0.125-8 | 0.5 | |
| 0.4 G | 2 | 2 | 4 | | 2 | 10 | |
| 0.5 G | 17 | | 0.5-16 | 1.8 | 1.8 | 0.5-4.0 | 0.25-2 0.125-1.0 |

From these estimations Heilman *et al* recommended a dose of 0.4 to 0.5 G every 6 hours. In 1953 however, Mathieu de Fossey drew attention to the fact that gastric juice reduced the activity of the drug while Josselyn and Sylvester (1953) observed that, although the drug was readily absorbed from the upper part of the gastro intestinal tract gastric secretion materially lessened the amount absorbed. When a crystalline preparation of the drug was given in suitably coated tablets the blood levels following oral administration were consistently within the range necessary to inhibit most susceptible organisms *in vitro*. This effect was confirmed by Smith, J. W., Dyke, and Griffith (1953) who found that gelatin coated capsules containing between 300 and 500 mg of the drug produced serum concentrations of over $1 \mu\text{g}$ per ml in the majority of patients and in fasting healthy subjects. Even the capsules, however, did not protect the drug completely during its passage through the stomach. Kirby, Maple and O'Leary (1953), using doses of 0.3 and 0.5 G in capsules found a concentration in the serum of less than $1 \mu\text{g}$ per ml in a third of their subjects. Tablets were then used which had a thin coating of an acid resistant material which disintegrated in media of pH 7.0 or more. With these tablets a striking difference in the blood

levels was observed after allowance had been made for the time taken for them to pass through the stomach. The following figures were obtained from assays made by a modification of Rammelkamp's method using as test organism a streptococcus inhibited by 0.02 μg per ml of erythromycin.

| Dose | No. of estimations at each interval | μg per ml at hours after dose | | | | |
|-------|-------------------------------------|--|-------|------|--------|--------|
| | | $\frac{1}{2}$ | 1 | 2 | 4 | 6 |
| 0.3 G | 4-6 | 0.0-2 | 0.0-5 | 0-2 | 1-4 | 0.4-2 |
| 0.5 G | 9-10 | 0.0-5 | 0-10 | 0-20 | 0.1-10 | 0.2-10 |

When doses were repeated at 6 hourly intervals the concentrations found were higher still.

| Dose | No. of estimations at each interval | μg per ml at hours after last dose | | | | |
|--------------|-------------------------------------|---|--------|--------|--------|--------|
| | | $\frac{1}{2}$ | 1 | 2 | 4 | 6 |
| 0.3 G 6 hrly | 10-13* | 0.5-20 | 0.5-20 | 0.4-20 | 0.5-10 | 0.2-10 |
| 0.5 G 6 hrly | 12† | 1-20 | 4-50 | 4-40 | 1-20 | 1-20 |

* Estimations made on the 2nd to 12th day after the beginning of administration.

† Estimations made on the 3rd to 16th day after the beginning of administration.

Thus it is seen that, once the dose had been repeated several times, blood concentrations within each 6 hour interval could reach the level required to inhibit susceptible organisms *in vitro*, this being in the neighbourhood of < 0.009 to 3.12 μg per ml (see Table 1).

Another method of counteracting the inactivation of erythromycin in gastric juice was that of Sylvester and Josselyn (1953) who used an insoluble acid resistant salt of the antibiotic—erythromycin stearate. This they gave suspended in carboxymethylcellulose, in doses of 200 to 500 mg before and after meals to adults, and in doses of 1.5 mg per lb of body weight to children, either as a single dose before a meal or as 3 doses given at 4 hourly intervals. The absorption of this preparation was at least as good as from the large capsules used earlier. The emulsion was moreover easier to swallow. Clinical trials with a suspension of cinnamon flavoured erythromycin ethyl carbonate were made by Gattman and Rosenbaum (1954). These workers gave their child patients a higher dose (5 mg per lb of body weight) 6 hourly for 5 to 11 days. Vomiting occurred in 1 case but no other evidence of toxicity was found. The children all recovered from their infections, which were mainly respiratory.

An appraisal of the absorption of different preparations administered by mouth was made by Griffith (1955) using a method of assay similar to that employed by Kirby *et al* (1953). From the 30 subjects used in these trials a fair approximation of what might be expected from a given dose of erythromycin was obtained.

| Dose | Average μg per ml at hours after dose (approx)* | | | |
|-------|--|------|------|------|
| | 2 | 4 | 6 | 8 |
| 0.2 G | 0.4 | 0.45 | 0.12 | 0.08 |
| 0.3 G | 0.64 | 0.64 | 0.16 | 0.1 |
| 0.5 G | 1.28 | 1.4 | 0.64 | 0.24 |

* Taken from logarithmic graph.

These average levels are within the range found by Kirby *et al* (1953) in their subjects but are rather low, since only 0.5 G produced a concentration of more than 1 μg per ml. Griffith (1955) then made an attempt to find a

coating for erythromycin which would be effective in preserving the drug during its passage through the stomach. He used 3 different preparations and computed the average concentrations in the blood serum after a single dose of 0.3 G had been administered. The preparations used and the concentrations obtained were as follows:

| Preparation of erythromycin | Average μg per ml at hours after dose of 0.3 G | | | | |
|--|---|------|------|------|------|
| | 1 | 2 | 4 | 6 | 8 |
| Stearate with carbowax like coating | 0.18 | 0.34 | 0.2 | 0.8 | 0.04 |
| With enteric coating | trace | 0.09 | 0.24 | 0.2 | 0.12 |
| With thin coating of cellulose acetate phthalate | 0.2 | 0.64 | 0.34 | 0.12 | 0.06 |

Even with the most satisfactory preparation, the last average levels did not at any time reach 1 μg per ml. Similar experiments carried out by Josselyn, Endicott, and Sylvester (1955) showed that only after a dose of 0.5 G of the stearate by mouth did average levels exceed 1 μg per ml. These levels ranged from 2.56 μg per ml at 2 hours down to 0.32 μg per ml at 6 hours when the suspension of the stearate or film coated base was used. With a dose of 0.2 G, the same preparation produced average levels of 0.32 μg per ml 2 hours and 0.04 μg per ml 6 hours after the dose was given. Higher levels were obtained but more slowly with the base coated by some special preparation which was not described. It must, however, be remembered that repeated administration would be likely to keep the blood levels closer to the higher concentrations than to those found 6 hours after a single dose.

Intravenous administration

Serum concentrations were studied by Maple, O'Leary, and Kirby (1953) after doses of 0.25 G of erythromycin were given dissolved in 10 ml of a special diluent. The solution was administered to 23 adult patients as an infusion in at least 200 ml of a 5 per cent dextrose solution in physiological saline. A dose of 0.5 G of the drug was given in 30 minutes to 12 patients and 1 G of the drug in 6 hours to the remaining 11. In these 2 groups the blood concentrations of erythromycin were assayed by a method similar to that used in the trials of oral administration. The results were as follows:

| Dose | No of cases | μg per ml at hours from starting infusion | | | | | |
|---------------------|-------------|--|---------------|------|------|-------|-------|
| | | $\frac{1}{2}$ | $\frac{1}{2}$ | 1 | 2 | 4 | 6 |
| 0.5 G in 30 minutes | 12 | 20 | 7-20 | 2-10 | 1-5 | 0.4-2 | 0.2-2 |
| 1 G in 6 hours | 11 | | 1-5 | 1-5 | 1-10 | 1-5 | 2-10 |

The higher dose infused over 6 hours did not appear to raise the blood levels above those obtained from the lower dose until the end of the 2nd hour, and the levels obtained then were comparable to those obtained by giving 0.5 G of the drug by mouth every 6 hours in tablets with an acid resistant coating. Thus intravenous administration was not found to be much better than oral administration except in the speed with which the first dose reached the blood stream. Using a glucoheptonate salt of erythromycin, Griffith, Johnstone and Smith (1954) found they could produce much higher blood levels by injecting the drug quickly, that is over 4 minutes. About 41 μg per ml were present in less than half an hour after injection of 0.3 G, but this concentration dropped to less than 1 μg per ml during the following 6 hours. When a higher dose was infused Geraci, Martin, Nichols, and Larson (1954) found

that continuous administration of 2 G over 24 hours produced a fairly steady level in the region of 3.4 to 4.4 μg per ml. In spite of the relatively high dose, all patients even a child of 3, to whom the salt was administered, tolerated it well. In Griffith's hands (1955) the glucoheptonate salt of erythromycin also produced higher levels than the same dose given by mouth. Even 0.3 G produced 2.56 μg per ml for 2 hours, but this fell to 0.32 μg per ml after 6 hours.

Intramuscular administration

A special preparation of erythromycin was made available for intramuscular use at the time that Griffith (1955) carried out his trials. This preparation was tolerated for several injections, but later produced a dull ache at the site of injection. The blood levels were eventually comparable with those produced by intravenous injection, but at 2 hours after injection the blood levels were only half as high as after a similar dose given intravenously. Detectable concentrations persisted for 8 hours. Davis and Romansky (1955) obtained more satisfactory levels with this type of administration by using the diethyl carbonate salt of erythromycin. Using a method of assay similar to that employed by Kirby *et al.* (1953) and a dose of only 0.1 G injected deeply into the muscles of the thigh, these workers found that the average levels reached 2.7 μg per ml in half an hour and fell steadily to about 0.5 μg per ml by the 6th hour after injection. A successful clinical application of this method of administration was carried out by J. C. Peele (1957) who used it for acute infections of the upper respiratory tract. He concluded that slightly more injections of erythromycin were required to bring about recovery than of 300 000 Units of procaine penicillin. It would seem therefore that, if a suitable analgesic preparation could be made, intramuscular administration should be suitable for treating cases in which it is not known at the beginning of treatment how susceptible to erythromycin the infecting organism may be. At the time of writing, however, the Council on Pharmacy and Chemistry of the United States (1957) stated that intramuscular administration was still only considered justifiable for severe infections caused by β haemolytic streptococci, pneumococci and staphylococci in patients allergic to penicillin and whose infecting organisms were sensitive to erythromycin. Even among this group it should be reserved for patients for whom intravenous or oral medication was not feasible. To avoid the infliction of pain and induration the dose should be limited to 100 mg or 2 ml of a solution containing 50 mg per ml.

A comparison of the methods of administration was made by Grigsby, Johnson and Simmons (1953). Erythromycin in some form was administered to 64 patients in a single dose of 0.5 G by mouth, and to 15 patients in doses of 0.25 G intravenously or 0.12 G intramuscularly, either as a lactobionate salt or in propylene glycol. In these trials parenteral administration produced decidedly higher blood concentrations than a much larger dose given by mouth. The clinical results obtained with intravenous and intramuscular administration as used by Griffith (1955) appeared to favour the intravenous route. 11 out of 14 patients with susceptible infections recovered when given erythromycin intravenously, while only 9 out of 16 did so after intramuscular administration. Davis and Romansky (1955) claimed that all 12 of their seriously ill patients with infections susceptible to erythromycin responded to

intramuscular therapy In both series, however, there were staphylococcal infections which did not respond to treatment No record is given of the results of oral administration

Distribution in the body fluids and tissues

Experimental work in rats showed that erythromycin was not only absorbed after oral administration but was found within 2 hours, in order of concentration, in the liver, submaxillary glands, spleen, adrenals, lungs, and kidneys Large amounts were also found in the thymus, skin, muscle, reproductive organs, and the heart (Lee, Anderson, and Chen, 1953) It is of interest, although it is not without parallel, that while the serum level dropped fairly rapidly concentrations in the tissues decreased much more slowly Detectable concentrations were found in the brain at a considerably later time than in the other tissues examined These workers later noted that the high concentrations found in the liver were reflected in the large amounts excreted in the bile (1954) In man, when serum concentrations were only in the region of $0.5 \mu\text{g}$ per ml, as much as $183 \mu\text{g}$ per ml, were estimated to be present in bile collected by means of a T tube by Takimura and Lopez Beho (1955)

Assays have been made after oral administration of erythromycin of the levels of the drug in cerebrospinal, pleural, and ascitic fluid, in the thyroid gland, and in the placenta Grigsby *et al* (1953) and Heilman *et al* (1952) found detectable concentrations in cerebrospinal and ascitic fluids of man up to 8 hours after oral administration but these concentrations were always lower than those found in the blood plasma The concentration in the cerebrospinal fluid was, however, dubious and did not appear to increase when the meninges were inflamed

Urinary excretion was relatively high, the concentration in the urine being about 120 times that of the plasma 8 hours after a dose of 0.2 G had been given Takimura, Lopez Beho, and Sher (1955) injected erythromycin into sterile post pneumothorax effusions, and found that although assayable amounts were present in the serum for 3 days after the last dose, only about 5 per cent of the total dose was excreted in the same time Further assays of erythromycin in serum and bronchial secretions were made by Lopez Beho, Takimura, Fornatto and Hollinger (1957) With doses of 0.5 or 1 G given by mouth the concentrations in the serum reached their peak level before those in bronchial aspirates but they were maintained for a longer period in the latter A similar phenomenon was seen after intravenous administration After aerosol administration a reverse process was seen, higher levels were obtained first in the bronchial secretions but these fell off more rapidly than in the blood serum

Erythromycin was usually detected in the cord blood or placenta of pregnant women at term after several repeated doses of 0.2 to 0.8 G had been given (Heilman *et al*, 1952, Kiefer, Rubin, McCoy, and Foltz, 1955)

Brannick, Heilman, Nichols, and Martin (1955) measured the concentration of erythromycin in the thyroid gland removed at operation and the concentration in the serum at the same time Detectable concentrations were found in both thyroid and serum $2\frac{1}{2}$ to $2\frac{3}{4}$ hours after the last dose had been given

Administration of erythromycin in combination with other antibiotics

In the hope of enhancing the activity of the antibiotic against a specific organism or of delaying the appearance of resistant strains, numbers of workers have used a combination of erythromycin with some other antibiotic. There seems little evidence from experiments *in vitro* that the first aim can be achieved against streptococci or staphylococci. Jones and Finland (1956, 1957 *d, e, and f*) could find no evidence of synergic effect in the sera of subjects administered erythromycin together with penicillin, chloramphenicol, tetracycline, oleandomycin, or spiramycin. Erythromycin administered alone produced concentrations inhibiting test organisms which were clearly superior to any combination.

Complications of therapy

Kaipainen and Faine (1954) found that guinea pigs and hamsters given doses of 33 mg of erythromycin per kg of weight by mouth or intraperitoneally began to die in 6 to 8 days. Apart from this, little evidence of toxicity from the administration of erythromycin has been encountered.

Haight and Finland (1952 *a*) treated 41 patients over 2 to 14 days with erythromycin. The majority of these received 0.1 to 2.5 G by mouth 3 hourly after an initial dose of double this amount. Only one case, with bacterial endocarditis, suffered from intractable vomiting after the dosage had been raised to 0.5 G 3 hourly. This patient died from endocarditis a few days later. The clinical findings of Haight and Finland thus confirm the experimental results of McGuire *et al* (1952). Heilman *et al* (1952) claimed that gastro-intestinal irritation was often experienced when the dose was raised to over 0.4 to 0.5 G 6 hourly, but Grigshy *et al* (1953) encountered no evidence of toxicity in 38 patients treated by mouth or intravenously with doses ranging from 0.1 to 0.4 G every 4 to 6 hours. With intramuscular injections these workers observed some induration of the soft tissues at the site of injection. There are, however, few reports of clinical trials in which not a single patient suffered from some gastro-intestinal upset.

The possibility that erythromycin might have been responsible for the production of agranulocytosis was raised by Wooley and Saslaw (1955). This patient had had a breast abscess following delivery of a child 3 weeks previously. The leucocyte count rose to 19 650 per cu mm, 86 per cent of the cells being neutrophils. After incision and drainage of the abscess triple sulphonamide tablets containing sulphadiazine, sulphamerazine, and sulphamethazine were administered until 12.2 G had been taken. At the same time erythromycin was given to a total of 14.7 G. Ten days later the patient complained of a sore throat; her temperature rose to 105° F, and both her pharynx and tonsils were seen to be covered with a greyish white pellicle from which a *Staph aureus* was cultivated. The white cell count had fallen to 950 per cu mm and no granulocytes were visible. The *Staph aureus* was eventually found to be resistant to penicillin, which the patient was then receiving in doses of 1 000 000 Units 3 hourly together with tetracycline but it was sensitive to erythromycin. Finally folic acid and streptomycin were given and the breast was again incised and drained. The leucocyte count then steadily rose; neutrophils appeared in the blood within 3 days and reached normal numbers after 2 weeks. It is of course, possible that the agranulocytosis was due to erythromycin, but this is made unlikely by the fact that

the patient received a second course of erythromycin (0.5 G 4 times a day for a week) without ill effects.

The gastro-intestinal upsets which often follow doses above 0.4 G 6 hourly necessitate suspension of the drug before more serious complications can occur.

Other complications due to the appearance of infections caused by organisms resistant to erythromycin are dealt with in the section devoted to specific infections.

Clinical trials

The results of a number of miscellaneous clinical trials were published between 1952 and 1954 for example, those of Grigsby *et al* (1953), Haight and Finland (1952a), Heilman *et al* (1952), Martin, Nichols, and Geraci (1953), Schwarzer and Ellenberg (1954), Shoemaker and Yow (1954), Tunnell and Hedenius (1954), and Solomon and Johnston (1955). From these studies it was clear that pneumonia and bronchitis, when due to the pneumococcus or haemolytic streptococcus, responded well to the administration of erythromycin, as did streptococcal infections of the nose and throat, staphylococcal infections of the alimentary canal, erysipelas and septicaemia due to susceptible organisms, while *C. diphtheriae* disappeared from the throats of carriers. Crises of urinary tract infection and empyema also responded when the organisms involved were susceptible to erythromycin, but again the outcome in staphylococcal infections was uncertain. Meningitis did not always respond satisfactorily, although 1 case due to staphylococcal infection was adequately treated by Herrell, Nichols and Martin (1953). A case of meningitis due to pneumococcal infection did not recover until penicillin was administered, possibly because of the uncertainty of the passage of erythromycin into the cerebrospinal fluid (Grigsby *et al* 1953). There were some bacterial infections where response was particularly uncertain, irrespective of the route of administration or the dose up to 0.4 G 6 hourly, for instance otitis media due to *Alcaligenes faecalis*, gonorrhoea, and bacterial endocarditis. It is doubtful whether recovery from bacterial endocarditis depended only on whether the infecting organism was initially susceptible to erythromycin or not. Haight and Finland (1952a) had no success in 2 cases infected with staphylococci, in both of these resistance to erythromycin developed. Similarly in the cases of Shoemaker and Yow (1954), staphylococcal infections did not improve under treatment. Heilman *et al* (1952), however, claimed a recovery without relapse in 1 case of endocarditis caused by *Str. salivarius*. In 2 other cases due to streptococci the blood stream was not cleared of organisms, and in one of these due to *Str. mitis*, considerable resistance to erythromycin developed.

In treating children Schwarzer and Ellenberg (1954) used a powdered form of the drug mixed with ice cream or apple sauce, or administered the antibiotic in tablets coated with an acid resistant substance. A dose of 12.5 to 25 mg per kg of body weight was given daily to children who were able to swallow the tablets, and 50 mg per kg of body weight to infants. The results obtained were similar to those already quoted. Pneumococcal pneumonia and acute bronchitis responded well, the temperature fell to normal in 1 to 3 days. Pneumonia due to *Haemophilus influenzae*, on the other hand, was more variable in its response. Similar results were seen in bacterial

infections of the upper respiratory tract and in otitis media, but, again, a case of bacterial endocarditis due to a staphylococcus was not improved by administration of the antibiotic.

Conclusion

From the figures quoted above it seems reasonable that a dose of 0.3 G. of erythromycin stearate in a thin enteric coat of cellulose phthalate should, when repeated 6 hourly, maintain high enough levels in the blood to inhibit organisms susceptible to less than $1 \mu\text{g}$ per ml *in vitro*. Undoubtedly in the individual case, the dose should be raised, even at the cost of producing gastrointestinal symptoms, if there is any doubt about the susceptibility of the organism concerned. Intravenous infusion should be reserved for patients who are either in coma or very seriously ill. Doses of 1 to 2 G. per 24 hours should be sufficient until the patients are well enough to accept oral administration. Intramuscular administration should be avoided whenever possible until such time as a preparation is available which induces less pain than do present ones.

THE TREATMENT OF DISEASES DUE TO SPECIFIC ORGANISMS

Staphylococcal infections

Since the incidence of staphylococcal infections resistant to penicillin and other antibiotics has been increasing, these infections have constituted the main field for therapeutic trials with erythromycin. Even in the earliest trials (Haight and Finland, 1952a and Heilman *et al.*, 1952) there was evidence not only that resistance was induced *in vitro* in staphylococci by erythromycin, but that organisms in closed lesions, for example in bacterial endocarditis, also became resistant to the drug.

Various trials have been made of the sensitivity of staphylococci to erythromycin *in vitro* and these all indicated that organisms inhibited by $1.0 \mu\text{g}$ per ml or less were widely distributed. Among the various workers who tested strains of staphylococci were the following*:

| Reference | No. of strains tested | Sensitivity <i>in vitro</i> (μg per ml) | Average |
|---|-----------------------|---|---------|
| Fusillo, Noyes, Pulaski, and Tom (1953) | 109 | 0.05-0.4 | |
| Grigsby, Chappelle, and Peacock (1953) | 146 | 0.078-0.625 | |
| | | 1.2-2.5 | .. |
| | | 5.0-20 or more | |
| Haight and Finland (1952a) | 614 | 0.01-1.56 | 0.39 |
| Heilman <i>et al.</i> (1952) | 30* | 0.2-2.0 | 0.4 |
| Hobson (1954) | 213 | 0.25-0.5 | . |
| Kutscher <i>et al.</i> (1954) | 30 | 'very sensitive' | . |
| McGuire <i>et al.</i> (1952) | 3 | 0.4-0.8 | . |
| Powell <i>et al.</i> (1953) | 4 | 0.39-0.78 | . |
| Thomson, Rountree, and Freeman (1956) | 26 | 0.15-1.25 | . |
| Tunevall and Hedenius (1954) | 127 | < 0.04-5.0 | .. |

* Of 120 strains later studied by Needham and Nichols (1953), none was found naturally resistant to erythromycin.

When, however, these staphylococci were exposed to increasing concentra-

tions of erythromycin all workers concurred that resistance developed Fusillo *et al* (1953) observed a 125 to 500 fold increase in resistance in a very sensitive staphylococcus submitted to 10 subcultures. Similarly resistance developed rapidly in the 14 strains studied by Hobson (1954) and the 25 tested by Grigsby *et al* (1955). Haight and Finland (1952 *c*) threw some light on this phenomenon by showing that variants with high resistance although not found in small volumes of cultures when first exposed to erythromycin soon appeared in subcultures containing gradually increasing concentrations of the antibiotic. In spite of showing no noticeable change in colonial morphological and most biochemical characters the resistant strains of *Staph aureus* lost their ability to produce coagulase. In addition to confirming these observations Hobson (1954) also noted that resistant strains were not limited to any particular phage type. It was thus clear that clinical trials of the drug would have to be approached with considerable caution. Although an occasional case of staphylococcal infection had been treated in series of miscellaneous infections Herrell, Nichols and Martin (1953) were the first to study a whole series of infections due to the staphylococcus alone. Erythromycin was given to 54 separate patients with staphylococcal infections resistant to penicillin, chlortetracycline and oxytetracycline. The dose given was 0.3 to 0.4 G administered by mouth 6 hourly. Although results were excellent in some cases they were unsatisfactory in others. This might have been ascribed to the method of administering the drug and the uncertainty that blood levels were above those required to inhibit the organisms. On the other hand the development of resistance was also a possible source of failure. In summary the results were as follows:

| Condition treated | No. of cases | Result of treatment |
|---|--------------|--------------------------|
| Septicaemia | 8 | Favourable response in 6 |
| Skeletal or soft tissue infection | 17 | 8 |
| Ileocolitis | 14 | 14 |
| Stool carriers | 5 | 5 |
| Miscellaneous for example meningitis urinary infections respiratory infections | 10 | 10 |

The uncertainty of the response was exemplified in the cases of septicaemia. Favourable responses were obtained in the following cases: infection of the elbow with surrounding cellulitis (1); the mastoid after mastoidectomy (1); the prostate after transurethral prostatectomy (2: 1 after a second course of erythromycin); and the skin following bullous dermatitis (1). Of the 2 patients who did not recover 1 suffered from septicaemia following a transurethral operation for carcinoma of the bladder and the other had bacterial endocarditis. In both of these cases the staphylococci became resistant to erythromycin. It is however curious that in a patient who was first treated with erythromycin for septicaemia after catheterization of the bladder and again when septicaemia followed a transurethral prostatectomy the organisms did not become resistant to the drug. The most interesting result was the unfailing response in cases with ileocolitis and in gastro-intestinal carriers of staphylococci. Although the ileocolitis in most of these cases was a sequel to chlortetracycline or oxytetracycline therapy and this condition is known to clear up spontaneously after discontinuance of the exciting drug the disappearance of staphylococci from the faeces of carriers following erythromycin treatment is not so readily explained. Moreover serious cases of

ileocolitis do occur when it is impracticable to leave the patient to recover from his infection without treatment. In 14 such cases erythromycin was of benefit (Herrell, Nichols, and Martin, 1953). Response to this condition, however, was not invariable. Frame and Short (1955) described a case of staphylococcal enteritis induced by an operation for a ruptured appendix and tetracycline. The staphylococci isolated from this patient's faeces and throat were identical and sensitive to erythromycin, yet the giving of this antibiotic in doses of 0.4 G 6 hourly together with supportive measures had no effect on the course of the disease, which ended in death. At autopsy a staphylococcus was isolated from the intestinal contents which was still sensitive to 0.3125 μg per ml of erythromycin although highly resistant to the tetracycline used in the test and relatively so to other antibiotics. Other instances where erythromycin was not invariably effective in bringing about recovery were 1 case among 3 treated by intramuscular injection by Perner and Puderbach (1956) and another among 6 all of whom were gravely ill before receiving erythromycin (Thaysen, Erksen, Knudsen, and Neukirch (1956).

Investigations by Kirby, Forland, and Maple (1953) on the use of erythromycin in infections resistant to penicillin produced results similar to those obtained by Herrell *et al* (1953). In some cases which were treated by surgery at the same time, for example, osteomyelitis and soft tissue infections, healing took place in 2 weeks, but in others, for example a patient with staphylococcal pneumonia, and another with osteomyelitis, erythromycin had no effect. In yet another case the organisms became resistant to the drug after 2 months' therapy. Whether the principal cause of failure in those cases which did not respond to erythromycin was the acquired resistance of the staphylococci or the failure of the antibiotic to gain access to the lesions must remain in doubt.

Another series of 26 patients with staphylococcal infections requiring surgery were studied by Pulaski and Wesolowski (1954). All these received erythromycin and, provided prolonged treatment was not required, the results were good. For instance, of the 2 recalcitrant cases in 12 with soft-tissue infections, 1 had chronic furunculosis of the buttocks, for which erythromycin was administered for 1 month. At the end of this time the staphylococci were resistant to the antibiotic. The second case was one of pustular acne for which, again, treatment was likely to have been prolonged. In 8 staphylococcal bone infections erythromycin appeared to have cleared up the infection in 5 cases, although these had previously been subjected to surgery. Of the 3 cases in which the infection did not clear up, 2 had followed fenestration operations in which some dead bone had presumably been left behind in the area of operation, and the 3rd case had a chronic osteomyelitis of the tibia with sinus formation. To those who have worked with penicillin it is common knowledge that it is impossible to clear up an infection where some dead tissue remains. With erythromycin the same should be true, with the added difficulty that prolonged treatment is likely to induce resistance in the infecting organism. A similar circumstance may also explain why a case with an empyema improved but did not cease to drain, staphylococci persisting in the drainage fluid. However, a satisfactory response was obtained with septicaemia following a generalized dermatitis, and in another where pericholangitis followed cholecystectomy and drainage of the common

bile duct by means of a T-tube. Both of these conditions were presumably acute and were treated at an early stage before any irreversible damage had been done to the tissues involved. The same might apply to a case of staphylococcal gastro enteritis which had failed to respond to penicillin, chlortetracycline, oxytetracycline, or streptomycin. The pure culture of *Staph aureus* obtained from the stool was resistant to all of these antibiotics but sensitive to erythromycin. With this drug the infection quickly responded and the patient was taking a regular diet on the 4th day of treatment, cultures of the stools showing a return of coliform flora.

An interesting result of the use of erythromycin in a maternity unit in Edinburgh was reported by Torfar, MacCabe, Balf, Wright, and Gould (1955). In this hospital all clinical infections in the newborn were treated with this antibiotic by mouth, 20 mg per lb of body weight being given daily in 6 hourly doses for an average of 4 days, although some cases received treatment for as long as 9 days. In another maternity hospital all similar infections were treated with erythromycin together with streptomycin injected in a dose of 0.125 G daily. These routine treatments were continued over a period of 9 months. By the end of that time in the first unit, where the babies had received erythromycin for any clinical infections believed to be staphylococcal, 114 swabbings from 140 different cases revealed an incidence of 74 per cent with coagulase positive *Staph aureus*, and 18 per cent with other staphylococci. In the second unit the proportionate incidence of coagulase positive and other staphylococci was much the same as in the first group. All the strains of staphylococci in the first unit were inhibited by 0.5 G of erythromycin per ml. The relatively minor infections treated in both units responded satisfactorily, except for 9 cases of conjunctivitis, yet no strains resistant to erythromycin emerged. It is possible that local instillations might have had a better effect on the cases with conjunctivitis as it is not known whether erythromycin is excreted in the tears. Moreover, continual reinfection from the conjunctival sac may have counteracted the antibacterial action of the antibiotic in the substance of the conjunctiva. In these early and superficial lesions—pustules, local cellulitis, or paronychia, infections of the umbilical stump or conjunctivitis—the failure of resistant strains to emerge was explained by the workers as due to the restricted use of erythromycin, only babies who showed some clinical evidence of infection were treated. The short time needed for treatment, an average of 4 days, may also have had something to do with the absence of resistant strains, but even this explanation cannot always hold good. Fullerton and Smith (1956, 1957) described 2 cases of staphylococcal septicaemia accompanying pneumonia in which serial tests revealed the emergence of resistance in organisms within as little as 5 days in one and in less than 3 weeks of erythromycin treatment in the other. How serious the problem of resistance may be was described by Lepper, Moulton, Dowling, Jackson, and Kofman (1953 c). In one contagious diseases hospital, except for chlortetracycline erythromycin was the only antibiotic used for a period of 5 months in all wards containing patients with pertussis or suspected coccal infections. Before this routine was introduced no strain of staphylococcus isolated from nose or throat cultures had required more than 0.98 μg of erythromycin per ml for inhibition. During the following 5 months a progressively increasing percentage of staphylococci were found in nose and throat cultures of patients and staff which were only

inhibited by 100 μ g per ml or more. Eventually 75 per cent of all staphylococci isolated required 100 μ g per ml for inhibition. Once erythromycin was withdrawn from use the proportion of resistant strains diminished until not more than 29 per cent were isolated 4 months later. Staphylococci which had an intermediate degree of resistance had by then almost completely disappeared. A second investigation carried out at the same hospital revealed that the prophylactic use of erythromycin for preventing the appearance of staphylococci in the trachea of tracheotomized patients suffering from poliomyelitis had been effective in over three quarters of the patients, but after 3 months' use of the antibiotic less than half of these patients could be protected in this way. Contrary to the findings in this epidemiological study was the report of Schneerson (1955) of the incidence of strains of staphylococci sensitive to erythromycin in 1953 and in 1954 at Mount Sinai Hospital, New York. In 1953 erythromycin was not widely used, but by 1954 it was generally available in the hospital. In 1953, of 1,164 strains isolated from various pathological sources, 93.6 per cent were sensitive to erythromycin, and in 1954, of 299 strains similarly isolated, 90.6 per cent were still sensitive. This small increase in resistant strains may be regarded as a tribute to the restraint with which the antibiotic was employed in this hospital. It must be remembered, however, that only strains obtained on primary isolation were included; there is no record of the incidence of those which became resistant under treatment. Another attempt to discover whether organisms initially sensitive to erythromycin became resistant under treatment was made by Clapper, Seerest, and January (1957). One hundred and fifty patients suffering mainly from upper respiratory tract infections were given short courses of treatment lasting between 4 and 12 days. Before treatment began and at a later date cultures were made of throat swabs and discharges. A very slightly greater proportion of bacteria, including *Str. viridans*, *Haemophilus influenzae*, and staphylococci, were found to be resistant at the end of treatment than were so before it began. If indeed resistance to erythromycin, as described by Lepper *et al.* (1953-4), is due in part to the development of resistance in the original infecting strains, it would seem feasible to attempt some combination of therapy so as to prevent it. With this end in view Coleman, Gunnison, and Jawetz (1953) found that, among other bacteria, *Staph. aureus* was strongly inhibited by simultaneous exposure to erythromycin and penicillin or streptomycin: the antibiotics usually showed some additive action and when given together reduced the frequency with which organisms became resistant. This, however, was not the case when erythromycin was added to chloramphenicol, chlortetracycline, or oxytetracycline. These conclusions were not fully supported by Benigno, Berti, and Cama (1954) or by Manten (1954). The findings reported by these workers were neither in full agreement with those of Coleman *et al.* (1953) nor with one another. The explanation possibly lies in differences in experimental methods, for instance, differences in the size of the inoculum used. Future experience will no doubt show whether or not this combination therapy inhibits the appearance of resistant strains. That continued treatment is not alone responsible can be inferred from the high carrier rate found in the staff as well as patients by Lepper *et al.* (1953-4) after extensive use of erythromycin. Further evidence of the passage of a single resistant strain from one patient to another irrespective of treatment was provided by MacCabe and Gould (1956) who so traced it by means of

bacteriophage typing. At the present moment it would seem that erythromycin should be strictly limited to accessible lesions which are resistant to other antibiotics.

Streptococcal infections

In the main, streptococci have been found to be particularly susceptible to erythromycin, especially β haemolytic streptococci. One would expect therefore that streptococcal infections would respond well to erythromycin therapy. Streptococcal infections of the respiratory tract, of burns, of other soft tissues, and those associated with scarlet fever have been studied. Haight, Ziegler, and Kahn (1954) studied the effect of erythromycin in 114 patients with respiratory infections, presumed to be streptococcal in origin. The effect of therapy was assessed by the clinical state of the patient, the presence and subsequent disappearance of Group A streptococci, and the formation of anti-streptolysin O. Erythromycin was given to patients in a daily dosage of 0.8 G for periods of 3, 5, or 7 days. These 3 periods were given to the patients in rotation in order of admission. The immediate effect of each type of therapy seems to have been much the same: the temperature and leucocyte counts fell, and the duration of the illness was reduced. There was also a marked decline during treatment in the number of cultures positive for Group A streptococci, but the number of positive cultures again rose after treatment had ceased on the 3rd or 5th day and increased steadily until 75 per cent of all cultures were positive by the 21st day after therapy. Patients who received erythromycin for 7 days were nearly all rid of their streptococci during treatment, but 13 per cent of them again showed positive cultures by the 21st day after therapy. The formation of antistreptolysin O was suppressed by the early administration of the antibiotic, and this suppression was greatest in the patients who received treatment for the longest time. With a daily dosage of 0.8 G of erythromycin, gastro-intestinal reactions were few and mild, and therapy did not have to be discontinued. It was therefore considered advisable to employ a higher dose, and to continue it for at least as long as the longest period for which the drug was given in this study.

Streptococcal infections in burns. These were studied by Lowbury and Cason (1954). The doses of erythromycin given were relatively low: 0.6 G daily for patients over 6 years and 0.4 G for those less than 6 years, usually for 6 days. The results were compared with those in another group of patients who were treated alternately with chlortetracycline or with erythromycin. *Str. pyogenes* was found in swabs of the burned surfaces in 4 out of 17 of 24 cases of burns within the first 3 days of treatment. Later than this, or at the end of treatment, no streptococci could be cultured from any of the burns. (The findings were similar for chlortetracycline.) Reappearance of streptococci after treatment ceased did, however, occur in three cases. Apart from these cases, 7 others who were infected with chlortetracycline-resistant strains of streptococci were treated. In all 7 the *Str. pyogenes* disappeared from the lesions. Regular swabbing of burned surfaces enabled these workers to recognize the presence of pathogens before their presence was clinically manifest. However, their figures tended to show that, even with antibacterial therapy, grafting was not as effective when *Str. pyogenes* was present as when it was absent.

Soft tissue infections. Three patients with acute streptococcal infections,

starting from foci in one of the extremities and involving lymphangitis and lymphadenitis, were given erythromycin by Pulaski and Wesolowski (1954). All responded well to erythromycin, and treatment was discontinued after 6 to 9 days. Three other acute infections due to *Str. pyogenes* were treated by Heilman *et al.* (1952) and all recovered uneventfully. The experience of Romansky, Nasou, Davis and Ritts (1957) with 5 patients suffering from streptococcal infections was encouraging. Those with pharyngitis due to group A β haemolytic streptococci even when accompanied by bacteremia, recovered. Two patients with bacterial endocarditis, however, were not so successfully treated. One with an infection caused by a β haemolytic streptococcus died from congenital heart failure without any evidence of infection at death. The other infected with a *Str. faecalis* did not respond to treatment till penicillin and streptomycin were substituted for erythromycin.

Streptococcal infections in scarlet fever Haight (1954) investigated the effect of erythromycin on 208 patients suffering from scarlet fever. These patients were divided into those receiving penicillin, those receiving erythromycin and those given only a placebo. The results were assessed in terms of the duration of the fever, leucocytosis, and rash, and of the presence or disappearance of Group A haemolytic streptococci in throat cultures. There was little to choose between the effect of penicillin and of erythromycin but there was a considerable difference between the effects of the antibiotics and those produced by the placebo. The formation of antistreptolysin O was largely suppressed by the antibiotics compared with that in patients receiving the placebo and suppurative complications of the disease were prevented by the antibiotics. There were however some disadvantages in the antibiotic therapy: rashes and urticaria accompanied the administration of penicillin in a good proportion of cases and gastro-intestinal disturbances appeared in a few of the patients receiving erythromycin.

Prophylaxis of streptococcal infections

In an attempt to see whether erythromycin might prevent the superinfection of streptococcal infections in children predisposed to upper respiratory tract infections, Tidwell and Lewis (1957) prescribed erythromycin stearate 100 to 125 mg as a syrup or tablet once daily to a group of children from the latter part of 1953 until June 1955. Whenever a cold developed the parents were instructed to quadruple the dose. These children, who numbered 48 by the end of the investigation, reported at monthly intervals when throat cultures were taken. During the time of study β haemolytic streptococci were isolated 8 times only (initial cultures had shown them to be present in 3 cases). The fear that regular administration of erythromycin might induce resistance in any staphylococci present in the throat led these investigators to compare the sensitivity of those isolated during the 1st and 2nd years. Though fewer strains were sensitive to erythromycin in the second year, the difference was so slight as not to have significance. Nevertheless this may have been the first sign of a trend which could become more serious as time progressed.

Pneumococcal infections

In most patients with pneumonia treated by erythromycin the disease was pneumococcal in origin. The pneumococcus is particularly sensitive to erythromycin and it was therefore expected that the results would be good.

The effect of erythromycin was compared with that of penicillin by Austrian and Rosenblum (1953) in 50 patients with pneumonia from whom a pneumococcus had been isolated and in whom the radiological pictures showed that the pneumonia was lobar in its distribution. Twenty four of these patients received erythromycin in doses of 0.4 G 6 hourly and 26 received penicillin in doses of 300 000 Units every 12 hours by intramuscular injection. There was little difference in the results from the two types of therapy, clinical recovery of the survivors took much the same time in each group and complications occurred with equal frequency. Two deaths occurred in the erythromycin group and 1 in the penicillin group, but one of the patients who died in the erythromycin group was an octogenarian and a diabetic. The only side effect noted was 1 case of drug fever in the erythromycin group. It must be remembered in considering these results that they were also a comparison between two different routes of administration. The variability of the absorption of preparations of erythromycin given by mouth is not necessarily greater than the variability of the absorption of newer preparations of penicillin given by mouth (for example, phenoxymethylpenicillin), but there is no doubt that the higher blood levels produced by intramuscular injection of penicillin did not materially improve the results. This is understandably so, for the extreme sensitivity of the pneumococcus to either antibiotic ensures that antibacterial concentrations will be achieved in the blood at least after each occasion that one or other is administered. Austrian and Rosenblum (1953) drew attention to the production of strains resistant to erythromycin *in vitro* although no report of clinical failure due to this cause has yet appeared. Drug resistance should however, be suspected in any case where the results of treatment with erythromycin are not entirely satisfactory.

A less satisfactory assessment of the value of erythromycin in pneumococcal pneumonia was made by Bunn and Cook (1953). Even though only 12 cases were treated with doses similar to those given by Austrian and Rosenblum (1953), i.e. 1.2 to 2 G daily for 1 to 12 days, only 5 patients had an uninterrupted recovery. In 4 resolution was delayed for over 3 weeks, another failed to respond to treatment until penicillin was given, and the remaining 2 died. These unsatisfactory results led Bunn and Cook (1953) to conclude that erythromycin should not be used in the treatment of pneumococcal pneumonia. Survival from this serious disease depends on gaining proper access to the pneumococci within the alveolar walls. Here as elsewhere they are often embedded in masses of fibrin so that recovery is always problematical. In 7 cases treated by Romansky *et al* (1957) 5 died within the short time of 30 hours from the beginning of treatment.

Diphtheria and carriers of *C. diphtheriae*

Since *C. diphtheriae* is the most sensitive of organisms to erythromycin diphtheria should be expected to respond to therapy. An early report by Haight and Finland (1952 a) claimed that *C. diphtheriae* could be eliminated from carriers by moderate doses of erythromycin. Blute (1954) treated 3 cases and 1 carrier with erythromycin and eliminated *C. diphtheriae* in all 4 after 24 hours treatment. Further evidence that erythromycin could eliminate this organism from the throat was given by Blake (1954) who quoted 3 patients, 1 of whom showed evidence of having had diphtheria due to a virulent mutis strain. In spite of parenteral and local administration of

penicillin, and later removal of the tonsils, the organism persisted in the nose and throat. After more than 3 months as a persistent carrier of *C diphtheriae* the patient was given 0.3 G of erythromycin 6 hourly for 10 days. Swabs were negative in 48 hours and did not become positive again during the following month while the patient was under supervision. Two children found to be carrying *C diphtheriae* during the course of a routine examination were also rid of the organisms within 3 days of beginning erythromycin treatment. Forbes (1954) was able to demonstrate the disappearance of *C diphtheriae* in 14 cases in which infection had persisted after 2 separate periods of treatment with penicillin. Erythromycin was administered for 5 days and cultures taken from the throat 4 days after treatment had ceased. No virulent organisms were then cultured. These records demonstrate the ability of erythromycin to eradicate *C diphtheriae* from the throat. They do not give any indication of the therapeutic value of the drug during the active phase of the disease. An attempt to assess this was made by Beach, Gamble, Zemp, and Jenkins (1955). These workers tried out the antibiotic on 43 young patients and 5 carriers. To these erythromycin was given as the ethyl carbonate, administered in amounts of 25 to 50 mg per kg of body weight per day divided into 4 to 6 hourly doses. To 10 of these patients 20,000 to 40,000 Units of antitoxin also were given intramuscularly. These workers concluded that *C diphtheriae* was eradicated from the nose and throat of all 48 subjects in an average of 2 days in cases with active disease and in 3 days in carriers. It was claimed that the antitoxin was necessary to control the manifestations of the disease. The ability of erythromycin to control an epidemic was demonstrated by Wood and O Gorman (1957) and Wood and Hemphill (1957) in both a boarding school and a hospital for mentally deficient inmates where 58 and 34 carriers were discovered in each institution following attacks of mild tonsillitis and death from acute respiratory obstruction respectively. Isolation of carriers and 10 days' treatment with 200 mg erythromycin 6 hourly to children, 300 mg 6 hourly to adults, together with disinfection of blankets and bedding of all carriers, served to wipe out the epidemic within 8 weeks. During the subsequent 7 months 1 only of the treated carriers again had a positive swab. The effect of erythromycin thus seems to be similar to that produced by penicillin by which *C diphtheriae* was eradicated from the nasopharynx in 75 per cent of patients in 3 to 5 days. Nevertheless it is valuable to have a second line of defence for those patients in whom the organisms are not eliminated by penicillin.

Whooping cough (Pertussis)

Since *H pertussis* had been shown to be highly susceptible to erythromycin *in vitro* (see Table 1) clinical trials of its efficacy against whooping cough were made. Brem and Kabling (1955), between June 1953 and January 1954, tested the drug in 27 children of 6 months to 7 years of age. The children were given 4 doses a day amounting to 50 mg per kg of body weight and the treatment was continued over 4 to 21 days. The preparation was tolerated well by the children and even when vomiting occurred soon after a dose had been given it could quite readily be given again. Brem and Kabling could see little change in the clinical course of whooping cough as a result of this treatment, and in 1 child *H pertussis* was isolated from a nasopharyngeal swab even on the 5th day of treatment. It should be pointed out, however,

that there were no controls in this series. No deaths occurred but in the case where the disease was most dangerous, a baby of 5 months, human hyperimmune pertussis serum was also given. Quite a different conclusion was reached by Mitolo (1955) who treated 18 patients of similar age at the University Paediatric Clinic, Genoa. Although the drug was given in a lower dose, 30 to 40 mg per kg of body weight per 24 hours over 10 to 12 days or as an aerosol containing 100 mg twice a day, Mitolo considered that the effect of the treatment was good and without side effects. Recovery took place in 10 to 20 days. As in the previous report there seems little justification for this conclusion either.

Venereal diseases

Gonorrhoea

The concentrations found to inhibit the gonococcus *in vitro* were low (see Table 1), and clinical trials were consequently begun by Gable, Romansky, and Taggart (1953). These workers found that 11 strains of *Neisseria gonorrhoeae* tested *in vitro* were inhibited by as little as 0.1 to 0.4 µg per ml of erythromycin. It was also found that 96 per cent of the patients treated by these workers with 2 G of erythromycin by mouth either in 1 or in divided doses, recovered from their infection. Fewer recovered on lower doses. Following these satisfactory results Alexander and Schoch (1954) treated 10 males suffering from acute anterior gonococcal urethritis with lower doses of erythromycin ranging from 0.5 G for 2 doses to 0.2 G 4 times in the day for 5 days. These workers saw no improvement in any of their patients. Again Manning, Jones, and Bigham (1954) treated 62 men with acute gonococcal urethritis with 0.5 G of erythromycin 6 hourly for 1 or 2 days. Although the results were not quite so successful as those of Gable *et al.* (1953) 58 (90 per cent) of the patients recovered the single day a treatment being at least as effective as that given for 2 days. In an attempt to reduce the dose of erythromycin Marmell, Shidlovsky, and Prigot (1955) gave a triple sulphonamide together with the antibiotic. Nevertheless it was not until the gradually increasing dose of erythromycin had reached a total of 2 G and the dose of the triple sulphonamides had been reduced to 3 G that the highest proportion of recoveries was recorded.

Finally a comparison was made between the effects of erythromycin, oxytetracycline, and tetracycline in gonorrhoeal urethritis by David (1955). The antibiotics were administered as follows:

Erythromycin—enteric coated tablets given in divided doses amounting to 3 G

Oxytetracycline—in capsules or tablets also in divided doses amounting to 3 G

Tetracycline—1.5 to 4 G in tablets or capsules given 4 times a day

The results in terms of immediate recovery and gastro intestinal reactions are set down below:

| Antibiotic | No. treated | Recovered | | Percentage with gastro intestinal reactions |
|-----------------|-------------|-----------|------------|---|
| | | No. | Percentage | |
| Erythromycin | 54 | 35 | 65 | 67 (the majority mild) |
| Oxytetracycline | 11 | 10 | 90 | 45 |
| Tetracycline | 89 | 87 | 96 | 35.9 |

These results, although possibly from not giving erythromycin in the ideal way, yet indicate that it is not so safe or sure a method of treatment as that with tetracycline

The effect of erythromycin was tried on women by Rubin, Somerson, Smith, and Morton (1954) who had observed that, although penicillin treatment usually cleared the gonococcus from the lower genital tract, some of the patients subsequently developed chronic pelvic inflammatory disease. More over, the isolation of pleuro pneumonia like organisms from the lower genito urinary tract became less frequent as gonorrhoea or other pelvic inflammation was cleared up. These workers treated 24 ambulatory negro women suffering from gonococcal infection, the nature of which had been confirmed by the isolation of the gonococcus from the cervix, with doses of 0.3 G 4 times a day until a total of 3.6 G had been given. Following treatment, cervical cultures taken at weekly intervals for 3 successive weeks showed that the gonococcus had disappeared in 22 of the subjects. The acid clinical test of an antibiotic is in its effect on bacterial endocarditis. On p. 29 can be seen the chart of a patient with acute gonococcal endocarditis who recovered on treatment with erythromycin alone. One can infer, therefore, that gonorrhoea will yield to erythromycin once a suitable dosage scheme is found.

In view of the frequency of gastro intestinal symptoms in these trials it would seem advisable to confine the administration of erythromycin to those cases where other chemotherapy has been unsuccessful.

Syphilis

In 1953 Keller and Morton tested the susceptibility to erythromycin of 3 strains of cultivable treponemes. These were the Kazan, Nichols, and Reiter strains. The sensitivity of these strains was assessed against that of the streptococcus C 203. The streptococcus was inhibited by between 0.05 and 0.1 μg per ml but the treponemes were inhibited by 0.01 to 0.05 μg per ml. These organisms were thus more sensitive to erythromycin than was the streptococcus, but they were less sensitive to erythromycin than to penicillin. This evidence of the responsiveness of cultivable treponemes to erythromycin led to clinical trials of the antibiotic in syphilis. Four cases of early syphilis were treated by Alexander and Schoch (1954). These were given 0.2 G of erythromycin in capsules 4 times a day for 8 days. Three of the patients became darkfield negative in 24 hours and 1 in 30 hours. The lesions, primary and secondary, healed promptly, but it was too early to make any statement about the effect of treatment on serological tests.

Granuloma inguinale

This venereal disease was first treated with erythromycin by Robinson and Cohen (1953). Nine patients who all had clinical evidence of the disease and in whom Donovan bodies were found in marginal scrapings, were given varying doses of erythromycin from 0.1 G to 0.3 G 6 hourly. Irrespective of the size of dose, complete healing followed in all but 1 patient who left the hospital when his lesion was showing every sign of healing. Alexander and Schoch (1954) tested the effect of erythromycin on 2 cases. The diagnosis was confirmed in each of these by the finding of Donovan bodies in smears. As they had done in the case of syphilis, these authors treated the disease with 0.2 G of erythromycin 4 times a day for 8 days, but they saw no improvement.

in the lesions over this time. The lesions subsequently responded to streptomycin.

Lymphogranuloma venereum

Alexander and Schoch (1954) treated 4 male patients with enlarged non-suppurating inguinal nodes with doses similar to those they had given for granuloma inguinale and syphilis. Again, they saw no benefit from this treatment. In 1955 an attempt to potentiate the action of erythromycin by giving triple sulphonamide was reported by Marmell and Prigot. The preparation was given in tablets, each containing 0.1 G of erythromycin and 0.83 G of sulphadiazine, sulphamerazine, and sulphamethazine. Two tablets were given 4 times a day to 4 patients in whom the diagnosis had been confirmed by a positive Frei test. In all cases rapid improvement in the inflammation followed, with subsidence of pain within 3 days. The lymph nodes ruptured in 3 cases during the course of treatment, which lasted for 3 to 10 days. The ruptured nodes exuded a thin watery discharge, but regressed and then healed in 3 to 5 days additional treatment. One lymph node resolved without rupture, and lesions of the penis healed after 5 days' treatment. The report was made too early to record whether any recurrence had occurred.

Conclusion

In conclusion it might be said that erythromycin, although often valuable in gonorrhoea, is uncertain in its effect when given in doses which do not produce gastro intestinal upsets. The evidence indicates that syphilis is particularly susceptible to this antibiotic, but further work and a longer period of examination of the patients' serological reactions are necessary before it can be stated that the antibiotic is a good substitute for penicillin. In granuloma inguinale the response is generally rapid and, provided that treatment is continued for long enough, healing occurs, but whether this is complete or not appears to depend on the size of the lesion. In lymphogranuloma venereum the results are equivocal, since no benefit was seen in cases treated with erythromycin alone.

Meningococcal infection

Anderson (1953) described a child of 2 years with meningococcal septicaemia treated by erythromycin. Following a cold of 2 weeks' duration this child became drowsy, began to vomit, and developed purpuric spots. The temperature rose to 104° F and convulsions occurred while the patient was being examined. Meningococci were isolated from the blood but not from the cerebrospinal fluid. The organisms were found to be resistant to penicillin, oxytetracycline, and chlortetracycline. In spite of sodium sulphadiazine and various antibiotics including penicillin, chloramphenicol, and streptomycin, either singly or in combination with one or other, the child improved only gradually, and relapsed with a high temperature on the 12th day after admission. By this time sensitivity tests had been carried out by the disk method with polymyxin and erythromycin. Although the meningococci were resistant to the former antibiotic they were found to be highly sensitive to erythromycin. This drug was then given in doses of 0.2 G 6 hourly for 8 doses and then 0.1 G 6 hourly, treatment being continued for

11 days Three days after erythromycin treatment was begun the patient was afebrile for the first time since admission, and the leucocyte count fell to normal Within 6 days the child was playing Although there were some neurological sequelae in the form of choreo athetoid movements of the extremities, these improved during the 3 weeks of convalescence before discharge and at the time of discharge the child was able to stand Four patients with this infection were treated by Romansky *et al* (1957), 1 of whom was suffering from a Waterhouse-Friderichsen syndrome By means of intravenous therapy to start with, followed by doses of 400 mg 4 hourly by mouth, 3 of these patients recovered The 4th, although she had responded well to therapy, eventually died from what was considered to be a pulmonary infarct

Actinomycosis

Four patients suffering from this infection were treated by Herrell, Balows, and Dailey (1955) *Actinomyces israeli* had been isolated from all the lesions and this was found to be inhibited by 0.25 to 0.5 μ g of erythromycin per ml In 2 patients the jaw was involved, the bone being invaded in 1 of them The 3rd case had actinomycosis in the perianal region, and the 4th had a swelling over the junction of sternum and clavicle Erythromycin was administered by mouth in doses of 0.3 G every 6 hours for 3 to 7 weeks The patient with bony involvement, however, was given penicillin after the course of erythromycin Drainage of abscesses, excision of necrotic tissue, and removal of a bony sequestrum were also carried out All 4 patients fully recovered, although it is difficult to ascribe to erythromycin alone all the beneficial effects which were said to be due to the chemotherapy In the case where penicillin was given after erythromycin the *Actinomyces israeli* isolated at the end of the course of erythromycin was still sensitive to 0.4 μ g per ml of this antibiotic

Brucellosis

Three reports of cases of brucellosis treated by erythromycin appeared in 1954 and 1955 Bearing in mind the variable susceptibility of the different types of *Brucella* to this antibiotic, very successful results were hardly to be expected from low doses of the drug Cassano, Miano, Barletta, and Mazzeo (1954) treated 8 patients infected with *Br. abortus* or *Br. melitensis* These authors claimed that all the strains were sensitive to erythromycin *in vitro* and gave their patients 0.3 G every 3 hours, later reducing the frequency of administration to every 6 hours The early results were good fever subsided in 3 to 4 days, blood cultures became negative, the patients' general condition and blood picture improved, and the erythrocyte sedimentation rate returned to normal Late results, however, were not so satisfactory, for relapse occurred in 5 cases The relapses were overcome with the help of intravenous vaccine and erythromycin again While not recommending this antibiotic as the first choice for treating brucellosis, these workers considered that it was of value in patients who had become intolerant to other antibiotics Contrary to the findings of investigators in the United States, León and Cano (1954) found that 0.03 μ g per ml was sufficient to inhibit all 3 species of *Brucella* which they tested Moreover the survival of chick embryos infected with *Br. melitensis* was prolonged for 3 days or more, even when treatment was begun 24 hours after they had been infected León and

Cano (1954), using much the same dose as Cassano *et al* (1954), 0.6 G 6 hourly, found that their patients had recovered in as little as 2.5 days, but the majority relapsed in 1 to 20 weeks after treatment ceased. When streptomycin was added to the treatment relapses were at least delayed, for no relapse was seen in patients who were observed for 3 to 9 months after treatment. Finally, Urteaga, Larrea, and Calderon (1955) treated 14 patients, 7 in the chronic stage of brucellosis. Diagnosis was confirmed in each case by positive cultures and agglutination tests. Erythromycin was administered as tablets in 3 doses daily amounting to 1.2 to 2.4 G until a total of 20 to 30 G had been taken over 9 to 16 days. Two or three times the higher dose was given over 2 to 3 days to some patients with similar results. In the acute cases, in which symptoms had been present for not more than 30 days, symptomatic improvement followed treatment as quickly as in the 2 previous series of cases, and little sign of intolerance to the drug was seen. In the chronic cases, who had been ill for 2 to 6 months or even 2 years, clinical improvement was evident within the first 2 days of treatment, and the temperature fell shortly afterwards. However, foci in the joints and spleen persisted, as did the leukopenia and disturbance of bone marrow function. In 12 cases who were followed up for some time, 5 showed relapses of fever, positive blood cultures and agglutination tests, but each exacerbation of the disease responded again to erythromycin, so that the attack in each case was limited to 24 to 48 hours. These relapses occurred irrespective of the size of the dose given, although it was hoped that by lowering the dose until it was just sufficient to overcome the fever the immune mechanisms of the host might be allowed to function in the normal way.

Amoebiasis

In 1953 McCowen, Callender, Lawhs, and Brandt confirmed the earlier findings of McGuire *et al* (1952) that *E. histolytica* could be cleared from infected rats by feeding them erythromycin in a total dose of 500 mg per kg of body weight.

Intestinal amoebiasis

Shafai (1955 *a* and *b*) made a clinical trial of erythromycin in 22 patients with intestinal amoebiasis in Egypt. Thirteen of these had had recurrent attacks for 6 or 7 years. Diarrhoea with blood and mucus, colic or abdominal pain, or localized tenderness over the colon or iliac region were present. Amoebae or cysts were found in the stools and mobile amoebae were found in mucus aspirated during sigmoidoscopy. Shafai gave his patients 0.8 G of erythromycin daily. After 10 days, 10 cases had shown prompt relief of diarrhoea and other symptoms, and intestinal ulcerations healed within 2 weeks. In 5 of the cases, however, cysts persisted in the aspirated mucus for approximately 3 weeks. These latter cases were subsequently cleared by carbasone, with or without erythromycin. In 7 patients who also had schistosomiasis ulcers due to the schistosomes persisted after erythromycin had been given, but the scrapings were negative for amoebae. In 1 case a mass palpable at the junction of iliac and sigmoid colon, and thought to be an amoeboma, was reduced to half its size in 3 months and, by the time treatment for the schistosomiasis was completed, it had disappeared. Shafai

noticed little difference in the response to treatment between cases with mobile trophozoites in the stools and those with only cysts. Forty-two patients with intestinal amoebiasis were treated by McHardy, Browne, McHardy, and Ward (1955). They received the same daily dose as Shafar's patients and for the same time. The value of their study was in the length of follow up which was conducted. The patients' faeces were examined at intervals for 6 months after treatment. After the 30th day there were 5 relapses, after 60 days—3, and at 6 months—1. Another method of administration was adopted in 8 cases. These received erythromycin stearate in a suspension according to the same dosage scheme. In these patients treatment was less successful for *E. histolytica* persisted to the end of treatment in 2 and there was also a relapse after 30 days in 1 patient. The dose of this preparation was sufficient to produce nausea, abdominal cramps, mild diarrhoea or pruritus and during treatment in 6 of the patients. Seventy patients with chronic and 28 with acute amoebiasis were treated in La Paz, Bolivia, by Villarejos (1955). *E. histolytica* was found in the faeces of both chronic and acute cases. Villarejos administered erythromycin as tablets with an acid resistant coating in a dose of 0.8 G by mouth to start with, followed by 0.2 G every 8 hours until each patient had taken a total of 4.6 to 6.5 G. The faeces were examined from time to time during the 3 months after treatment. *E. histolytica* had disappeared from 67 cases of chronic amoebiasis by the end of treatment but after 3 months there had been a relapse in 8. Better results were obtained in those patients receiving 6.5 G than in those to whom only 4.6 G was given. In the acute cases rapid clinical improvement followed the beginning of treatment, stools being normal in number within 62 hours and no amoebae being found after 96 hours. One relapse only occurred, at the end of 3 months. With the doses employed in this series, no unfortunate side effects were observed.

Parenteral amoebiasis

When amoebae have passed through the intestinal wall and lodged in foci elsewhere the high concentrations produced in the intestinal contents by oral administration are no longer available to attack the protozoa. Nevertheless clinical trials were made to see whether it was still possible to influence the disease. Extra intestinal lesions were also present in 3 patients of Shafar (1955a) who had tenderness over the liver. These all cleared up while under treatment. Anderson, Nelson, Carbone, and Daar (1955) treated 45 patients in Sevilla, Columbia, who had evidence of hepatic involvement. Fifteen of these patients received erythromycin stearate as 15 mg per kg of body weight daily. Hepatic amoebiasis cleared in 14 of these patients, and in those in whom *E. histolytica* was demonstrable in the faeces, the amoebae had disappeared before treatment was completed. Nelson, Anderson, and Thomas (1955) then turned their attention to amoebic hepatitis in the United States. They found evidence of this in 29 out of 371 cases admitted to the Sonoma State Hospital, California. Like Shafar's 3 patients, none had a definite liver abscess but liver function tests and liver biopsies showed the presence of subacute hepatitis and abnormal liver function. These patients received 1 G daily for 14 days if adult and 15 mg per kg of body weight if children. In 20 of these patients the liver condition responded favourably, the remainder subsequently being treated with chloroquine. The results with erythro-

mycin, according to Shafar (1955 b), were similar to those obtained with fumagillin, streptomycin, bacitracin polymyxin, neomycin and diiodo hydroxyquinoline in 200 cases previously studied. A trial of erythromycin, given as 6 G over 10 days, together with fumagillin, produced no more striking improvement than erythromycin alone, given as 8 G over 10 days¹. However, with the two antibiotics given together, recurrences in the following 3 months were said to be less frequent. The combination of fumagillin and erythromycin produced no better results than did fumagillin with tetracycline.

Virus infections

Prime influenza

A total of 263 patients were included in a study by Cronk and Naumann (1954) on the effect of erythromycin on prime influenza. These patients were admitted to hospital between January and March 1953. A prime influenza virus was isolated from 82 per cent of them. When satisfied with the diagnosis, Cronk and Naumann (1954) divided these patients into 3 groups according to the treatment given: (a) 91 patients who received analgesics, (b) 61 patients who were given a placebo, and (c) 89 patients who received 0.2 G of erythromycin 4 times a day. In a third of the group who received only a placebo a secondary rise of temperature occurred on the 4th day in hospital, but this did not occur in any of the patients receiving erythromycin. In none of the groups was there any significant secondary infection. It was thus somewhat difficult to ascribe a therapeutic effect to erythromycin.

THE TREATMENT OF DISEASES CONSIDERED BY SYSTEMS

Infections within the Chest

Bacterial endocarditis

Mention has already been made of several cases of bacterial endocarditis treated with erythromycin. In none of those caused by a staphylococcus was treatment successful. One case due to *Str. salivarius* responded (Heilman *et al.*, 1952) but others failed to do so. From the same clinic Geraci and Martin (1954 a) found their results disappointing in a further 7 cases. In only the case infected by *Str. salivarius* was a good response obtained. Of 4 cases with staphylococcal infection 3 died within 36 hours to 9 days of beginning treatment and another died 2 months later—from cerebral embolism. Another patient was infected with an enterococcus and another with a *Str. mitis*. In the latter an initial response was obtained but the organism rapidly became resistant to erythromycin. The enterococcal infection showed no improvement under treatment. These last 2 patients were then treated with dihydrostreptomycin and penicillin and eventually recovered. Pulaski and Wesolowski (1954) treated a case of endocarditis due to α haemolytic streptococci

¹ For further information regarding fumagillin and erythromycin see fumagillin p. 159.

which were resistant to penicillin, chlortetracycline, oxytetracycline, and chloramphenicol, but sensitive to 0.4 μg per ml of erythromycin. In spite of maintaining concentrations of 1.25 to 2.5 μg per ml of erythromycin in the blood, there was no response and the patient died 19 days after treatment had been commenced. Many vegetations were found on the aortic cusps at autopsy. From studies *in vitro* Geraci and Martin (1954a) thought that a combination of erythromycin with bacitracin might be more effective than either antibiotic alone.

A better result was obtained by Lamhert, Lamalle, and Lievens (1955) in a non haemolytic streptococcal infection. This patient had been ill for a month, during which time no organism was isolated from the blood. Before the streptococcus was cultured penicillin and streptomycin in 'massive' doses had been administered intravenously. After these antibiotics had produced no obvious therapeutic effect, oxytetracycline was infused by the same route. When sensitivity tests were at last available it was found that the streptococcus was resistant to all 3 antibiotics used up to date but that it was sensitive to erythromycin. This was then given in doses of 2 G daily over 1 month followed by progressively decreasing doses during the next month. The patient responded well to this treatment, did not experience any side effects, and was eventually clinically cured. No recurrence followed the cessation of treatment over a period of 5 months. Fig. 1 is the chart of a patient who recovered from acute bacterial endocarditis due to a gonococcus particularly susceptible to erythromycin, which was the only antibiotic she received.

Combined therapy A combination of erythromycin with oxytetracycline had success in a staphylococcal infection described by Johnson and Hurst (1954) in a child of 3 years. Doses of penicillin up to 60 million Units daily by intravenous injection were given, together with intramuscular streptomycin, but this had no effect on the child's condition in 5 days. When erythromycin was substituted for the penicillin the fever fell, but blood cultures remained positive. When oxytetracycline was substituted for the streptomycin, however, there was an impressive clinical improvement and complete subsidence of fever after oral administration had taken the place of intravenous treatment. The child maintained his improvement and was regarded as having recovered from his infection. It is of interest to note that the 2 antibiotics to which the staphylococcus was most sensitive *in vitro* were oxytetracycline and erythromycin, each of which inhibited the organisms at 0.37 μg per ml.

Another report made by Bishop, Smith, Lloyd Jones, and Longcope (1955) described a case due to a non haemolytic streptococcus in a patient with a congenital defect. The illness had failed to respond to a succession of antibiotics including penicillin, streptomycin, neomycin, bacitracin, and oxytetracycline in combination with penicillin, even though blood levels of penicillin had been raised by means of probenecid well above those necessary to inhibit the organism. Eventually erythromycin, which inhibited the streptococcus *in vitro* at a concentration of 3 μg per ml, was added to the penicillin. It was administered intravenously together with heparin in doses of 2 and 14 G respectively each day by means of a polyethylene catheter introduced through the femoral into the iliac vein. When it was found that blood levels of 12 to 18 μg per ml of erythromycin were being maintained, the penicillin was discontinued. The blood cultures became sterile and no recurrence of the bacteraemia appeared after the treatment had begun. The

patient did not suffer further from symptoms of congestive heart failure up to 4 months after treatment, although she did complain of dizziness and ataxia.

It would seem from these accounts that erythromycin can rarely be depended on alone to control subacute bacterial endocarditis, but in combination with another antibiotic it may bring about recovery. Which antibiotic should

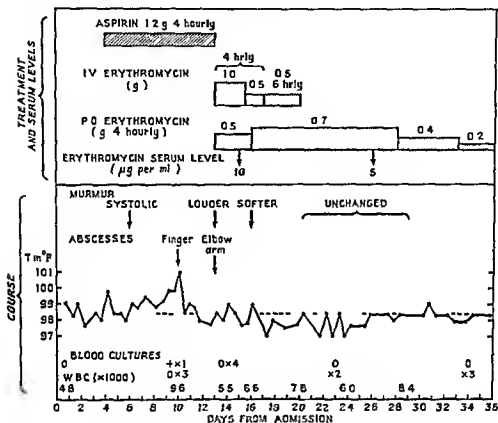


FIG 1 Chart of treatment and course of a case of acute endocarditis and arthritis due to *Neisseria gonorrhoeae* sensitive *in vitro* to 0.1 µg per ml of erythromycin and treated by this antibiotic. It can be seen that the serum levels during the first 2 weeks of treatment far exceeded the susceptibility of the organism *in vitro*. Erythromycin was continued for a further 9 days as 0.4 g by mouth every 6 hours. Ten months later the patient continued in excellent health.

(From Davis and Romansky *Amer J Med* 1956 21, 473)

be used in such a combination can only be indicated by sensitivity tests *in vitro*.

Pneumonia

The results of treatment of cases of pneumonia ascribed to the pneumococcus have already been described under pneumococcal infections (p 18). Those which were not selected according to their aetiological organism were investigated by Romansky, Nasou, Davis, and Ritts (1957). These workers treated 221 patients with pneumonia in 171 of whom the distribution was lobar, bronchial in the remaining 50. *Diplococcus pneumoniae* was cultured from the sputum in 73 instances and from the blood in 43. In a further 84

patients, although a bacteriological diagnosis was not obtained, the findings were 'typical of pneumococcal pneumonia'. Treatment consisted in administering 100 to 600 mg by mouth every 4 to 6 hours and by parenteral injection to 50 very seriously ill patients. With such presumptive evidence that the great majority of infections were pneumococcal in origin, the results are of interest. These were good in 93.2 per cent of cases. The 8 deaths in the series were attributed to insensitive infection—*Klebs pneumoniae*, to patients being moribund on admission, or to accompanying disease such as cor pulmonale, alcoholism, emphysema, and cirrhosis. With such results in an unselected series one would be tempted to think that Bunn and Cook (1953) had been unfortunate in having patients who were had risks under any form of treatment (p. 19), if the only further report available was that of Gibson, Nushan, and Anderson (1954). These latter workers, like Austrian and Rosenblum (1953), compared the results of therapy with erythromycin and with penicillin; 24 patients were treated with erythromycin and 21 with penicillin. The response was found to be similar in the 2 groups. These workers expressed the guarded opinion that the usefulness of erythromycin had yet to be tested in fulminating infections, and this caution was in part justified by the investigation carried out by Waddington, Maple, and Kirby (1954). This included 75 elderly patients, many of whom were alcoholics or debilitated from age or some other cause. It was therefore unlikely that these patients would recover spontaneously from an attack of pneumonia. Pneumococci were isolated from the sputum in 43 cases and from the blood in 11. Erythromycin was given in acid resistant tablets in doses of 0.3 to 0.5 G 6 hourly, sufficient, as Kirby *et al.* (1953) had already shown, to inhibit pneumococci. Although 63 of these patients did well, complications such as delayed resolution and sterile effusions occurred in 11 cases in spite of adequate dosage, and 3 patients died. Nevertheless in such patients the prognosis with penicillin treatment is not always good, and it does not seem likely that better results would have been obtained even if penicillin had been administered in the usual way, by intramuscular injection.

Empyema

This condition was tackled by Lopez Beho, Takimura, Fox, and Lees (1956). In all cases the staphylococcus was responsible and in 21 out of 86 patients it was resistant to penicillin. Lopez Beho *et al.* grouped their patients according to the presence or absence of a broncho pleural fistula, the presence or absence of external drainage, and according to whether their conditions were acute or chronic. Their treatment consisted in administering erythromycin intravenously as 0.25 G in a 1 per cent solution over 24 hours when patients were very ill and by mouth as they showed signs of recovery—0.4 G 3 times daily. When the solution was not too irritating to the patients, they installed it through a catheter or by means of needle and syringe when cavities were closed. With this treatment the exudate had diminished or ceased within 72 hours and the cultures had become negative. In chronic cases this was only a temporary phenomenon, however, but treatment put them into a good condition to stand operation.

Pericarditis

Treatment of a single case was described by Nieman (1957). This was in a

man of 49 years whose condition of fulminating staphylococcal septicaemia involved lungs, subcutaneous abscesses as well as the pericardium. After the staphylococci were found to be resistant to the patient's initial therapy—penicillin and streptomycin—he was given erythromycin by mouth. Unfortunately for the assessment of its value in securing the patient's recovery, erythromycin was not the only antibiotic administered. As the pericardial exudate was aspirated a solution containing chlortetracycline was instilled into the sac. After 9 such aspirations and instillations over 4 weeks the staphylococci at last disappeared. As the original sensitivity test showed the organism to be resistant to oxytetracycline, the probability that it was also resistant to chlortetracycline can be considered, the repeated aspirations and instillations acting as a modified form of drainage in assisting erythromycin to rid the body of its infection.

Urinary Tract Infections

The scope of erythromycin in urinary tract infections is necessarily limited since none of the Gram negative rods commonly associated with infections in this region is susceptible to its action. It was however, tried without success in 3 cases of non specific urethritis by Haight and Finland (1952 *a*) and later by Willcox (1954 and 1955). By 1955 Willcox had treated 53 patients: 4 negroes, 1 Ceylonese, and 48 white people. The urethral discharge was cleared up in 1 to 3 days in 22 of the patients, but in the others it continued for a week, or even for more than 2 weeks. Gonococcal infection was excluded in this series of patients, although 2 of them had positive Wassermann tests. Willcox gave his patients 0.3 G. of erythromycin 4 times a day for 5 days or until 6 G. had been given. Apparently the discharge and dysuria subsided in most cases, but when these patients had been followed up for 2 to 3 months 11 out of 50 failed to maintain their improvement. A similar proportion of failures was also recorded 1 month after treatment. Although it is possible that erythromycin may have had some effect on certain susceptible bacteria in the urethra, the results do not provide much evidence that it is a particularly useful drug for non specific urethritis. In the earlier series (1954) the drug was administered in doses of 0.1 G. 4 times a day for 6 days, but 7 out of 21 cases failed to be improved by this treatment.

Other urinary tract infections were treated by Pulaski and Wesolowski (1954). These authors chose 22 patients without prostatic hypertrophy, other urinary tract obstruction, or gonococcal infection. An excellent response was obtained in acute infections of the prostate or epididymis, but in chronic infections the response was not particularly satisfactory. An immediate improvement occurred in 5 out of 13 patients with chronic prostatitis, but erythromycin resistant organisms were found in the urine or the prostatic secretion of patients who did not respond to the antibiotic. Even in those patients who improved, recurrences were apt to occur. One patient who had pyelonephritis and glomerulonephritis and in whom a mixed infection of the urinary tract was present was given erythromycin together with penicillin and streptomycin. However, this patient died on the 5th day of treatment, probably from the glomerulonephritis.

Subscribing to the theory that erythromycin may be effective in infections whose responsible organisms are resistant *in vitro* to this drug Mouratoff,

Bell, and Batterman (1957) treated 32 patients suffering from chronic genito-urinary infections which had persisted for 1 month to several years. Though the organisms isolated from the urine were, in order of frequency, *Proteus vulgaris*, *A. aerogenes*, enterococci, *E. coli*, with an occasional growth of *Ps. aeruginosa*, haemolytic streptococci, *M. pyogenes*, *aureus* or *albus*, and *Str. pyogenes*, 'clinical control of these conditions was manifest in 27 patients, the criterion of responsiveness being subsidence of fever, disappearance of dysuria, frequency, and albuminuria. Mouratoff *et al.* stated that in 26 of these successful trials there had been no alteration in the Gram negative flora. In spite of these excellent results one would prefer to know for how long these patients were followed after their apparent cure and whether there was no possibility that the exciting cause of their original complaint could not have been an organism only intermittently appearing in the urine. To support this peculiar theory an exhaustive study is required of the relevant factors contributing to the conditions treated and to their alleviation.

Infections of the Eyes

Trachoma

Although trachoma responds readily to sulphonamides in the acute stage, when it is neglected and becomes chronic it is difficult to eradicate. Since the disease is due to one of the larger viruses there was some hope that erythromycin might have some effect on it. From the Navajo Indian Reservation in the United States Button (1955) selected 21 patients in all stages of the disease except the late inactive stage of scarring. Ten children in the group had been treated with sulphadiazine and local sulphathiazole for 12 days without showing obvious improvement, while the remainder had had no chemotherapeutic treatment at all. The diagnosis was confirmed by conjunctival scrapings and biopsy examination in all cases. Erythromycin was administered in doses of 2 to 3 mg per lb of body weight at 4 to 6 hourly intervals. The clinical effect was remarkable: inclusion bodies disappeared first, then the follicle cells, then the macrophages, and finally the lymphocytes and plasma cells. Even in the early cicatricial stage, when pannus formation was present, examination of the conjunctivae showed a normal cellular structure after an average of less than 6 days' treatment. Although the microscopic changes appeared promptly there was a certain lag before gross clinical signs of improvement were obvious. Follicles and pannus often persisted for some time after the cytology of the conjunctiva appeared normal. At least 12 days were required for cure. When treatment was discontinued after less than 12 days there were some recurrences. Some confirmation of these remarkable findings was given by Constantinovits (1956). His treatment, however, was by local application—an ointment containing 0.25 per cent of erythromycin was introduced into the conjunctival sac 3 times a day at first, and later, as improvement progressed, once a day. Constantinovits observed no evidence of irritation or hypersensitivity. Bacteriological examination produced no growth after 3 days' treatment and later follicle formation and papillary hypertrophy gradually subsided. Even in the presence of pannus favourable results were obtained. By treating one eye of each patient with oxytetracycline and the other with erythromycin this investigator could compare their effects. He does not seem to have considered

erythromycin inferior to oxytetracycline in this respect but he observed that recurrences were not very much reduced by either drug

Infections of the Skin

In 90 per cent of 184 pyogenic lesions of the skin Livingood, Head, Johnson, and Nilasena (1953) isolated staphylococci which were inhibited by concentrations of 0.1 to 2 μg of erythromycin per ml, and/or β haemolytic streptococci all of which were inhibited by 1 μg per ml or less. From only 10 per cent of the lesions were Gram negative rods cultivated. The results of local applications of erythromycin were satisfactory in 37 of the 41 primary bacterial infections, but in only 56 of 143 secondary infections.

A series of 10 children with pyoderma was treated by Freeman and Scott (1953). Four of these children received erythromycin both by mouth and as an ointment applied to the lesions, the remainder received the ointment only. No new lesions were seen after 24 hours of treatment. Crusts had formed and healing was taking place within 48 to 60 hours, and healing continued until treatment was stopped. No recurrences were seen while the children were under observation. There was little difference between the progress of the children receiving oral as well as topical administration and those having topical treatment only. Pyogenic infections supervening on such conditions as primary dermatitis, acne pustulosa or cystica, impetigo, ecthyma, and hydradenitis suppurativa, were treated by Luhowe (1954) with 1 per cent of erythromycin in a petrolatum base. This worker found the ointment particularly effective against pneumococcal and staphylococcal infections which were resistant to other antibiotics. Forty three out of 55 cases improved under this treatment. So far this is the only series in which an unfavourable side reaction to local application occurred, and this occurred in a case with a varicose ulcer. Luhowe (1954) also used the ointment as a post operative dressing in 22 cases after the removal of verrucae, seborrhoeic keratoses, naevi, or sebaceous cysts. Although all of these cases healed rapidly without secondary infection, this would in any case be expected in a surgical unit where proper attention was paid to aseptic technique. A larger series than those already described was that of Robinson, H. M., Zeligman, Robinson, R. C. V., Coheo, and Shapiro (1954). These authors treated 1014 cases by local application of 1 per cent erythromycin in a base of oil and soft paraffin. This application was found beneficial in various pyogenic infections including impetigo, ecthyma, and sycoosis as well as in secondarily infected conditions. Oral administration of erythromycin in doses amounting to 0.8 G daily was also used for a large variety of skin conditions. Good responses were obtained mainly in pyogenic infection, but these authors also believed that erythema multiforme responded to the treatment. Again no skin reactions were seen but, as in other series treated by the oral route, gastro intestinal disturbance developed in 34 patients.

A series of 14 children with pyoderma was treated by Laing and Scott (1954) with a combination of polymyxin and erythromycin incorporated in an ointment. All the organisms isolated from the lesions were staphylococci, and most of them were susceptible to 2.5 μg of erythromycin per ml or less. Streptococci were isolated in some cases, and all of these were inhibited by this concentration of erythromycin. Only an occasional staphylococcus was

found to be susceptible to both polymyxin and erythromycin. There is some difficulty in understanding the rationale of this combination, and the clinical trial showed that there was no apparent advantage in adding polymyxin to the erythromycin in the ointment. Healing was complete in all patients in an average of 4.7 days. With such rapid healing the risk of superinfection of Gram negative organisms susceptible to polymyxin must have been minimal.

Acne vulgaris

The previously mentioned papers have demonstrated the effectiveness of erythromycin, applied locally or administered by mouth, in checking pyogenic infections of the skin. Van de Erve (1954) attempted to see whether the antibiotic was effective in acne. Sixty cases, 10 to 35 years old, with acne showing comedones, oily skin, and some pustulation, were treated by mouth in doses of 0.2 G given in capsules 3 to 4 times a day. The pustular element of the condition was controlled in nearly all the patients, but the improvement was temporary, and pustules again appeared, usually within a month after treatment. There was little or no effect on the sebaceous secretion of the skin. Similar observations were made by Johnson, Schuster, and Grimstad (1957) whose 74 patients had varying degrees of infection accompanying their acne. A dose of 250 mg 3 times a day was continued for 3 weeks up to as long as 5½ months. An excellent response was seen in 46 of their patients. This involved the disappearance of erythema, small cysts, and crusting, whereas the comedones persisted. Though staphylococci were isolated from the cysts in every case, these workers could not associate their increasing resistance to erythromycin with poor clinical response.

Reference to the treatment of pyogenic infections of the skin not mentioned in the text: Schonberg (1953)

Miscellaneous Conditions

Leukaemia

Although there is no reason to suppose that erythromycin should have any direct effect on myeloblastic leukaemia, it was thought possible that it might be of value in tiding the patient over febrile crises which were perhaps due to infection with susceptible organisms. Three such cases of aleukaemic myeloblastic leukaemia were studied by Reich (1954). In all of these the temperature had risen to 100° F or over and there was a considerable fall in the number of cells in the blood, particularly in the number of neutrophils. Myelohlastic infiltration of the bone marrow was evident. Chlorotetracycline or oxytetracycline was administered to 2 of these patients but caused gastrointestinal disturbance after a week in 1 patient, a lower dose of the drug failed to control the fever. Erythromycin was then given 3 times daily in doses of 0.1 G together with blood transfusions and cortisone. This therapy controlled the fever temporarily, but all patients eventually died from their disease 5 to 18 months later. The advantage of erythromycin over the tetracyclines in these conditions was the relative ease of its administration and its apparent effectiveness in controlling the fever.

Infections of the mouth

Thirty three college students with painful and bleeding gums, acute ulcers

tion of the mucous membrane of the gingival margins, and fuso spirochaetes isolated from their lesions, were treated by Cronk and Naumann (1954). They received 2 capsules (0.2 or 0.4 G.) 4 times a day for an average of 48 hours, together with 800 mg. of vitamin C daily. The response to this treatment was very satisfactory in those who could be followed. They gave the figures shown below.

| <i>Dose</i> | <i>Total no patients</i> | <i>Total followed</i> | <i>No clinically cured</i> | <i>No bacteriologically cured</i> |
|-----------------------|------------------------------|---------------------------|------------------------------------|---|
| 0.4 G., 4 times a day | 24 | 19 | 19 | 14 |
| 0.2 G., 4 " | 9 | 9 | 6 | 4 |

The higher dose produced better results, but on the other hand it also induced gastro intestinal irritation during treatment.

CHAPTER 2

ANTIBIOTICS RELATED TO ERYTHROMYCIN BY REASON OF BACTERIAL CROSS RESISTANCE

CARBOMYCIN, SPIRAMYCIN, OLEANDOMYCIN

CARBOMYCIN

GENERAL CONSIDERATIONS

LIKE erythromycin, the antibiotic carbomycin (*Magnamycin*) was isolated in 1952 and has similar antibacterial properties. Tanner, English, Lees, and Routien (1952) described its isolation from *Streptomyces halstedii* which grows luxuriantly in submerged aerated culture. Carbomycin can also be obtained from several other strains of actinomycetes. It is a crystalline monobasic material only slightly soluble in water from which, however, acid salts can be prepared, such as the hydrochloride and sulphate, which are water soluble. The base was found to have no effect on coliform organisms in anything but very high concentrations *in vitro* but Gram positive bacteria such as streptococci, pneumococci, *Staph aureus*, and diphtheria bacilli were inhibited by 1.2 μ g per ml or less. A second variety of carbomycin was produced by Chas. Pfizer and Co. Inc. This was called carbomycin B to distinguish it from the original product, carbomycin A. It has similar antibacterial properties (see p. 37), but has advantages over carbomycin A when administered to man.

Antibacterial activity

The antibacterial activity of carbomycin according to the findings of various workers is set out in Table 2.

Its activity *in vivo* was demonstrated by Wong, James, and Finlay (1953) against the following rickettsiae and viruses in embryonated hens' eggs: *R. prowazekii*, *R. typhi*, *R. akari*, *R. tsutsugamushi*, *R. rickettsii*; rickettsia of N. Queensland tick typhus, *Coxiella burnetii*, and the viruses of psittacosis,¹ ornithosis, lymphogranuloma venereum,¹ human and feline pneumonitis, sporadic bovine encephalomyelitis. It was not effective, however, against the following viruses: herpes simplex, meningo encephalomyelitis, rabies, vaccinia, poliomyelitis—type II virus.¹

Experiments carried out in mice by English, Mullady, and Fitts (1953) showed that full protection was afforded by the drug against 3 different strains of *Staph aureus* resistant to other antibiotics available at that time, and against *Str. pyogenes* ATCC 8668, in doses of 20 to 50 mg per kg given by subcutaneous injection half an hour after intraperitoneal inoculation of the

¹ In hens' eggs only, not in mice.

test organism. With *Str pneumoniae* ATCC 6301 protection was not so complete but, according to these workers, it was greater than that given by half the dose of penicillin.

TABLE 2 CARBOMYCIN CONCENTRATIONS REQUIRED TO INHIBIT DIFFERENT BACTERIA IN VITRO

| Species | μg per ml for inhibition | Species | μg per ml for inhibition |
|--|-------------------------------------|------------------------------|-------------------------------------|
| <i>A. aerogenes</i> | 100-200 | <i>N. meningitidis</i> | 0.2-1.56 |
| <i>Br. bronchiseptica</i> | 0.25-12.5 | <i>Pasteurella multocida</i> | 0.007-10.0 |
| <i>Bacillus subtilis</i> | 0.19-0.78 | Pneumococci | 0.039-0.156 |
| <i>Candida albicans</i> | 100 | <i>Ps. aeruginosa</i> | 100->100 |
| <i>C. diphtheriae</i> | 0.78 | <i>Proteus</i> sp. | 100 |
| <i>Clostridia</i> (4 species) | 0.39-1.0 | <i>Salmonella</i> | 25->200 |
| <i>E. coli</i> and other Gram negative species | 3.12->100 | <i>Sh. sonnei</i> | >25 |
| <i>H. influenzae</i> B | 1.56-50 | <i>Staph. albus</i> | 1.25-6.2 |
| <i>H. pertussis</i> | 3.12 | <i>Staph. aureus</i> | 0.05-12.5 |
| <i>Klebs. pneumoniae</i> | 6.25->25.0 | <i>Str. faecalis</i> | 0.19-12.5 |
| <i>Lepto. icterohaemorrhagiae</i> | 10.0 | <i>Str. mitis</i> | 0.039-0.8 |
| Mycobacteria | 3.12->25.0 | <i>Str. viridans</i> | 0.3-12.5 |
| <i>N. catarrhalis</i> | 0.02-0.78 | <i>Str. pyogenes</i> | 0.015-1.25 |
| <i>N. gonorrhoeae</i> | 0.078-1.6 | Other streptococci | 0.1-12.5 |

From the data of Cook and Thompson (1957), English, Field, Szendy, Taghian, and Fitts (1952), Finland, Wilcox, Wright, and Purcell (1952), Fusillo, Noyes, Pulaski, and Tom (1953), Gorzynski and Neter (1953b), Tanner, English, Lees, and Routien (1952), and Welch, Randall, Reedy, and Kramer (1952).

Protozoa. Trials of carbomycin *in vitro* against *E. histolytica* were made by Seneca and Ides (1953). Six different strains were inhibited by concentrations of 31.25 to 125 μg per ml. At a later date Seneca and Bergendahl (1954) found that subcultures of a particularly hardy strain of *E. histolytica* could be maintained in subinhibitory concentrations of carbomycin until by the 24th subculture it grew well in concentrations of 25, 50, 100, and 200 μg per ml. Growth gradually diminished in subcultures containing 200 μg of the drug per ml but continued to be good in lower concentrations. *Trypanosoma cruzi* and *Leishmania donovani* were inhibited by 31.25 to 62.5 μg of carbomycin per ml but the effect of the drug against *T. rhodesiensis* was doubtful. Trichomonads were inhibited only at a concentration of 250 μg per ml.

Other antibacterial properties of carbomycin were investigated by English *et al.* (1952). The minimal inhibitory concentration of the antibiotic was little affected by different types of culture media, and the efficacy of the drug was not appreciably diminished by human serum in concentrations of 1 to 20 per cent. Under certain conditions carbomycin could be bactericidal, but 5 times the minimal inhibitory concentration was required to kill *Staph. aureus* or *Str. faecalis*, and 10 to 125 times as much was needed to kill saprophytic mycobacteria.

Acquired and cross resistance

English *et al.* (1952) observed that resistance developed *in vitro* in a stepwise manner resembling penicillin in this respect rather than streptomycin. Nevertheless the steps were not necessarily very small ones. Hsieh and Kotz

(1955), in testing staphylococci with various salts of carbomycin *in vitro*, found that though highly resistant mutants did not appear after the first culture, those resistant to 1,000 μg per ml and others dependent on carbomycin for growth could appear within 3 stages only. The effect on cultures of amoebae has already been described above. The early development of resistance and the fact that the drug was mainly bacteriostatic gave warning of possible difficulties in its clinical application. English *et al* (1952), studying cross resistance, isolated 35 different organisms from patients clinically resistant to treatment with other antibiotics. These organisms were resistant to all or some of the following antibiotics: penicillin, oxytetracycline, chlortetracycline, chloramphenicol, dihydrostreptomycin, bacitracin, and polymyxin B. Thirty four of these organisms were inhibited by 0.39 to 3.12 μg of carbomycin per ml. One only, which was resistant to more than 100 μg per ml of penicillin, streptomycin, and bacitracin, and to more than 50 μg per ml of oxytetracycline and chlortetracycline, required a somewhat higher concentration of carbomycin for inhibition—6.25 μg per ml. However, Kutscher, Seguin, Lewis, Piro, Zegarelli, Rankow, and Segall (1954) found that all of 265 strains of bacteria isolated from pathological material between April and December 1953 were equally sensitive or resistant to carbomycin and erythromycin. These findings were obtained whether or not the organisms had been exposed to one or other of the 2 antibiotics. Finland, Wilcox, Wright, and Purcell (1952) found that repeated subcultures of staphylococci or streptococci in carbomycin or erythromycin resulted in a fairly rapid and marked increase in resistance not only to the antibiotic to which the organisms were exposed but also to the other one. Similar results were obtained with staphylococci by Fusillo, Noyes, Pulaski, and Tom (1953). Nevertheless resistance to carbomycin developed more rapidly in staphylococci and some enterococci according to Hewitt and Wood (1953) than it did to erythromycin, and Hsie, Kotz and Nasser (1956) observed that 352 mutants resistant to erythromycin or carbomycin did not always manifest cross resistance to both antibiotics simultaneously.

Administration

Oral administration

Absorption of carbomycin into the blood stream after oral administration appears to be very uncertain. Bramerd, Kawata, and Scaparoni (1953) could detect not more than 0.2 μg per ml in the blood of patients given an oral dose of 2 G, and no detectable concentration was found in 5 out of 11 patients after 6 hours. These authors considered that this was due to most of the antibiotic being excreted in the bile, for in jaundiced patients a dose of 2 G produced blood levels of 2.8 to 5.25 μg per ml for 1 to 6 hours after administration. Later Kutscher, Piro, Zegarelli, Lane, and Seguin (1953 b) confirmed the fact that serum concentrations were very variable following oral administration. Hewitt and Wood (1953) found detectable concentrations in the blood in 80 per cent of cases given 0.5 G 6 hourly by mouth. With repeated doses of the drug Finland, Purcell, Wright, and Del Love (1953) found that the concentration in the blood was never higher than 0.5 μg per ml, and in nearly half the specimens assayed no carbomycin could be detected.

Intravenous administration

Following intravenous administration more reliable serum levels were found by Brainerd *et al* (1953). After a dose of 0.5 G intravenously blood levels were about 0.6 μg per ml 2 hours after injection, and 0.075 μg per ml 6 hours after injection. Hewitt and Wood (1953) found demonstrable concentrations in the serum following injections of 0.4 G.

Excretion of carbomycin

Very little of the antibiotic could be demonstrated in the urine by Hewitt and Wood (1953), only 0.1 to 1.3 per cent of the daily ingested dose being found. More was excreted after intravenous administration. The drug appeared in the urine within 30 minutes of injection and 2.5 per cent of the daily dose was recovered in the urine. In different patients treated with doses of 2 G daily by mouth the concentrations in the 24 hour specimens of urine varied between 0.625 and 20.0 μg per ml. The amount recovered after a single dose was more variable.

Kutscher, Piro, Zegarelli, Lane, and Seguin (1953 *a*) assayed the concentrations of carbomycin excreted at 1, 3, and 5 hours after injection, and found these varied from 0.2 to 8,000 μg per ml or more.

Distribution in the tissues

This was studied by English, Rapuzzi, Field, McNierney, and P'An in the rabbit (1954). Half an hour after intravenous injection of 100 mg of carbomycin per kg the drug was found widely distributed in all tissues examined, except in muscle, brain, and spinal fluid. In 3 hours the activity found in most tissues was equal to that found in the serum. After oral administration of 200 mg of carbomycin per kg, the drug was also found widely distributed in the body tissues, and at 2 hours more of the drug was found in the tissues than in the serum. This disparity increased with time.

In man little work seems to have been done on this aspect of carbomycin administration. However, Brannick, Heilman, Nichols, and Martin (1955) studied the concentration of the drug in serum and thyroid tissue. When carbomycin A was administered 3½ hours before removal of the thyroid gland no activity could be found in either the gland or the serum. On the other hand, when carbomycin B was administered serum levels of 0.1 to 1.3 μg per ml were detected between 2 and 10 hours after the last dose, and in 8 out of 10 samples of thyroid tissue there were 0.3 to 1.5 μg per ml.

Complications of therapy

This antibiotic was not found to produce toxic reactions either from acute or chronic dosage in various laboratory animals by Gardocki, P'An, Rapuzzi, Fanelli, and Timmens (1953). The LD_{50} in mice for carbomycin injected intravenously was 550 mg per kg weight, and dogs tolerated as much as 200 mg per kg weight given by mouth or intravenously daily for as long as 20 weeks.

In man, although toxic effects were not observed in early clinical work, there was no doubt that gastro intestinal upsets were frequent enough to cause investigators to keep the dose as low as possible. Nausea, vomiting

and diarrhoea were seen in a quarter of the patients treated by Hewitt and Wood (1953), mainly on a dose of 0.5 G 6 hourly by mouth, and in a similar proportion of patients by Finland, Purcell, Wright, and Del Love (1953) even though the latter workers gave half this dose at frequent intervals. Even in young men, with acute streptococcal infections for which treatment was not continued for more than a few days, some gastro intestinal symptoms appeared according to Manning, Jones, and Bigham (1953). Relief from these untoward effects was obtained by giving the antibiotic with milk or food but this entailed the risk of poorer absorption of the drug. Hewitt and Wood (1953) observed skin eruptions on some of their patients, but these were not severe or urticarial except in 1 patient with scleroderma and a past history of drug sensitization. Disorientation and somnolence were observed in 2 cases, but it is very questionable whether these symptoms were due to the antibiotic, for both patients were elderly and were at that time recovering from a severe pneumonia. Complete blood counts, bi weekly urinalyses, and weekly determinations of the van den Bergh reaction, the cephalin and thymol flocculation tests, the serum alkaline phosphatase activity, the urinary urobilinogen, the blood urea nitrogen, and the blood sugar revealed no abnormalities. There was no clinical evidence of toxic effects on the central nervous system or of changes in the reflexes.

Clinical trials

Early trials were undertaken by Bunn and Cook (1953), Finland, Purcell, Wright, and Del Love (1953), Hewitt and Wood (1953), Manning, Jones, and Bigham (1953), and Whitaker, Prigot, Marmell and Morgan (1953). From these it was concluded that, provided the relatively high oral dose of 0.5 G 6 hourly was given, pneumococcal pneumonia, streptococcal infections, and acute staphylococcal infections of the throat responded quickly in the majority of cases. The response in staphylococcal infections of the urinary tract or elsewhere was uncertain. Gonorrhoea only responded occasionally even when treatment was given by intravenous injection, but granuloma inguinale responded to carbomycin given by mouth or intravenously. Amoebiasis also responded to carbomycin and the stools of carriers were frequently cleared by the drug. In all these cases the efficacy of treatment seems to have been limited by the uncertain blood levels produced by the oral administration of doses which were calculated to produce a minimum of gastro intestinal disturbance.

THE TREATMENT OF DISEASES DUE TO SPECIFIC ORGANISMS

Streptococcal infections

These were studied in greatest detail by Manning *et al* (1953). Twenty-three young men between 18 and 32 years of age suffering from pharyngitis, exudative tonsillitis or scarlet fever were treated with 0.2 to 0.5 G by mouth 6 hourly. β haemolytic streptococci were first cultivated from the throats of these patients, but could not be cultivated 48 hours later in 18 of 22 cases. Nevertheless fever persisted in 2, and in one of these streptococci could again

be cultivated from the throat 96 hours after treatment was begun. In another case a relapse with fever followed after 4½ days of treatment. From these results it might be concluded that carbomycin was not so certain in its effects as penicillin.

Venereal disease

Gonorrhoea

No response at all was seen in patients treated with daily doses of 1 to 2 G given by mouth. This was observed not only by Whitaker, Prigot, Marmell, and Morgan (1953) but also by Manning *et al.* (1953). Intravenous administration of 0.5 to 1 G in 1 or 2 injections did, however, result in 3 of 23 patients being cured, a not very successful result in any case.

Syphilis

Eleven cases of early syphilis in both men and women were treated by Buckinger, Hookings and Garson (1955) with an initial dose of 1 G followed by doses of 0.25 G. The drug was given in sugar coated tablets which were taken until a daily dose of 2 to 3 G was reached. The whole course lasted for 5 to 7 days. Buckinger *et al.* noted that the treponemes disappeared from the lesions in 36 to 72 hours after the first dose and healing, which began before the treponemes had disappeared, was complete in 2 to 14 days. This report, however, was made too soon to provide any information about the serum reactions. A later report of Hookings and Graves (1956) however included the original 11 cases together with 29 additional patients also with primary, secondary, or early latent syphilis. They eventually arrived at a dose of 1 G initially followed by 0.5 G 4 times a day until a total of 41 or 42 G was reached. They summed up their later results as follows:

| Period being followed after treatment | No. cases observed | Serological test* | Condition of CSF† |
|--|-----------------------|--|-------------------|
| Primary syphilis | | | |
| 2 weeks-4 months | 12 | Progressing favourably in 4 negative in 8 | Negative in 7 |
| 4-6 months | 8 | Progressing favourably in 2 negative in 6 | Negative in 4 |
| 7-12 months | 9 | Progressing favourably in 1 negative in 8 | Negative in 3 |
| 13-24 months | 14 | Negative in 14 | Negative in 5 |
| Secondary syphilis | | | |
| 12-16 months | 22 | Negative in 22 | Negative in 6 |

* Serological test = the VDRL testing using cardiolipin antigen

† Criterion of activity = cell count exceeding 5 per cu. mm. total protein of more than 40 mg. per 100 ml. of fluid

All cases tested were negative by this criterion

Lymphogranuloma venereum

Whitaker *et al.* (1953) made a trial of carbomycin in 5 cases with genito-inguinal lesions and associated adenitis. These patients all had positive Frei tests and positive complement fixation tests for lymphogranuloma venereum.

Given by mouth or intravenously, carbomycin in doses of 0.5 G once or twice a day had no effect on the progression of the lesions

Granuloma inguinale

Although there was little experimental work to indicate that carbomycin would be of value in this disease, Whitaker *et al* (1953) gave the drug to 7 cases of this disease in the usual dose of 0.5 G intravenously or 1 G orally twice a day. The diagnosis in these cases was confirmed by finding Donovan bodies in scrapings from anal genital lesions. The patients did well, with both oral and intravenous therapy. In 5 cases healing had occurred before the patients were discharged and the remaining 2 cases left hospital voluntarily while healing was progressing. These satisfactory results from carbomycin therapy were confirmed by Robinson and Cohen (1954) in 6 patients whose lesions began to heal even with smaller oral doses of the drug—0.2 to 0.3 G 6 hourly. The time taken for healing to be completed varied with the extent of the lesion and was accomplished in 9 to 44 days.

Amoebiasis

It is doubtful whether carbomycin has a specific and lasting effect on amoebiasis. Hewitt and Wood (1953) found that amoebae disappeared from the stools of 5 cases under treatment with this antibiotic, and Sodeman and Jung (1953) claimed that carbomycin had a specific therapeutic effect on amoebiasis of the colon. Nevertheless the findings of Seneca and his collaborators (1953, 1954) lead one to suppose that the effect of the drug is likely to be short lived, since *in vitro* amoebae rapidly develop the capacity to multiply in increasingly high concentrations of the antibiotic.

THE TREATMENT OF DISEASES CONSIDERED BY SYSTEMS

Infections within the Chest

Bacterial endocarditis

Hewitt and Wood (1953) gave carbomycin to 6 patients suffering from bacterial endocarditis. Two of these were due to an enterococcus, 2 to a non-haemolytic streptococcus, and 2 to *Staph aureus*. Although the infecting bacteria were inhibited by 0.63 to 1.56 μ g of the drug per ml, the patients showed only a temporary, if any, response to carbomycin therapy. There were, however, complicating factors in 2 cases. One had a large mesenteric abscess in the lesser peritoneal sac, and another, with a staphylococcal infection, also had acute pyelonephritis with a blood urea nitrogen of 64 mg per 100 ml. Two patients responded temporarily, 1 with a streptococcal and the other with a staphylococcal infection, and, curiously enough, these 2 cases were caused by organisms which were more resistant to carbomycin than the others. Three of the streptococcal infections responded to large doses of penicillin alone or penicillin with streptomycin or terramycin, but the patient with the large mesenteric abscess and the one with pyelonephritis died in spite of the administration of these antibiotics.

Pneumonia

There were several early trials of carbomycin in pneumococcal pneumonia. Bunn and Cook (1953) treated 8 cases from whom pneumococci had been isolated, some of these were acutely ill and others critically ill. Doses of 1.25 to 2 G of the drug were given daily for periods varying from 1 to 12 days. Of the 8 cases, 4 made a satisfactory response but 3 showed no signs of responding and in 1 there was delayed resolution. Since pneumococci were amongst the most sensitive of organisms to carbomycin this did not look a very promising beginning. A careful study was also made by Finland *et al* (1953) in 40 cases of pneumonia who had not received any other antibiotics for their disease. In 31 of these the pneumococcus was isolated from the sputum. These patients received a somewhat higher dosage than that given by Bunn and Cook (1953). They were given between 1.7 and 4 G of the drug daily. Treatment was begun with 0.5 G and in an attempt to maintain measurable blood concentrations Finland *et al* (1953) continued with doses of 0.2 G 3 or 4 hourly. Nevertheless pneumococci were still present when the sputum was examined again some time after treatment had begun. Moreover 6 of the 7 patients who had positive blood cultures did not respond satisfactorily. Two of these died within an hour after therapy was begun, 1 received penicillin before the next culture was taken, and 3 still had positive cultures for 2 to 6 days after carbomycin was begun. Cultures of the sputum were also relatively slow in being cleared of pneumococci. In 17 cases in which the sputum was tested pneumococci were still present 3 to 5 days or more after the beginning of treatment. Finland *et al* (1953) also tested the sensitivity of the infecting pneumococci. All of 20 strains tested were inhibited before treatment by 0.1 to 0.8 μ g per ml, but in 1 patient from whom a pneumococcus was isolated 4 days after treatment began the inhibitory concentration had increased 4 fold. This increased resistance under treatment did not, however, apply to all strains. Finland *et al* (1953) came to the conclusion that the results of carbomycin therapy in pneumococcal pneumonia were distinctly inferior to those observed with any of the other antimicrobial agents currently in use at the time of reporting. Another early series of cases with pneumococcal pneumonia was studied by Hewitt and Wood (1953). These investigators gave 0.5 G of the drug 6 hourly, although they were aware that few of their patients would have much more than an inhibitory concentration in their blood stream with this dose. None the less 10 of their 12 patients did respond to treatment within 4 days. The acute symptoms and signs had disappeared within this time and pneumococci were no longer to be found in the sputum. This criterion was however, not strict enough for Finland *et al* (1953) who, from their previous experience with other antibiotics expected the fever to have subsided within 2 days. In studying these responses Field and Taylor (1954) wondered whether the result might be a question of dosage. They accordingly divided their 28 cases of pneumococcal pneumonia into 2 groups giving to half of them the dose generally prescribed by Hewitt and Wood (1953) i.e. 0.5 G 6 hourly, and to the other half an initial dose of 1 G followed by 0.25 to 0.3 G 6 hourly. Ten out of the 14 patients treated by the higher dose responded well. Four, however, did not appear to be recovering and it became necessary to administer penicillin. With the lower dose 10 patients also made a satisfactory response.

and 4 responded slowly. When 1 G 6 hourly was given to 1 of these patients the temperature fell rapidly. In assessing these results it should be borne in mind that the expected course of pneumococcal pneumonia in survivors untreated by chemotherapy is by crisis on the 3rd to 5th day of the disease. The periods quoted here were calculated from the first day of treatment.

Other types of pneumonia Several patients with pneumonia due to *H influenzae* have been treated with carbomycin. A case described by Hewitt and Wood (1953) responded rapidly after the institution of carbomycin treatment. In a patient treated by Finland *et al* (1953) the organism disappeared from the sputum within 2 days. It was quickly replaced, however, by a haemolytic streptococcus. It should be noted that although this organism was only found as the primary invader in 1 of the cases described by Finland *et al* (1953) it appeared in the sputum of no less than 10 patients from whom it was not originally isolated.

Carbomycin therapy was of no benefit in 2 cases of staphylococcal pneumonia treated by Hewitt and Wood (1953) and presumably in the case described by Finland *et al* (1953) in which *Staph aureus* persisted in the sputum.

Ten cases in which a variety of organisms was present in the sputum were treated by Finland *et al* (1953). The sputum in these cases contained *H influenzae*, β haemolytic streptococci, α haemolytic streptococci, *Staph aureus*, and/or *Klebsiella pneumoniae*. Of these patients about half seem to have responded to therapy. A case with *Klebsiella* in the sputum was treated by Field and Taylor (1954) with carbomycin but showed no clinical improvement under therapy. It is well to remember that examination of the sputum after carbomycin therapy had been begun revealed a number of bacteria which were not initially found. Thus, among 45 patients with pneumonia treated by Finland *et al* (1953) a variety of coliform bacilli appeared in 15, α haemolytic streptococci in 11, *H influenzae* in 10, enterococci in 3, *Staph aureus* in 3 β haemolytic streptococci in 2 and monilia in 1. Although not necessarily responsible for the pathological changes, each of these organisms is capable of becoming pathogenic under suitable conditions. They are therefore not to be regarded without concern when carbomycin therapy is employed.

Bronchitis

A prompt clinical response was observed in all of the 8 patients treated by Hewitt and Wood (1953), in spite of the fact that the sputa of most patients showed only normal throat organisms. In these patients the temperature subsided within 24 hours of beginning therapy.

Tonsillitis

Six cases were treated with carbomycin by Hewitt and Wood (1953). One with fusospirochaetal infection responded well, but 1 due to a pneumococcus did not. Of 3 from whom β haemolytic streptococci were isolated only 1 did well while there was no response in a case due to *H influenzae*. From these findings one might perhaps suppose that the patient with oral cellulitis treated so successfully with carbomycin by Kutscher, Piro, Zegarelli, Lane, and Seguin (1953 a) must have had an infection of fusospirochaetal type. The cellulitis had its origin in a tooth infection.

Soft-tissue Infections

Several staphylococcal infections of this sort, but not all, were treated satisfactorily by Hewitt and Wood (1953). A case described by Field and Taylor (1954), with an abscess of the buttock, responded well to carbomycin. One patient suffering from erysipelas and treated by Field and Taylor (1954) also responded satisfactorily to treatment.

Urinary Tract Infections

Groups of patients suffering from urinary tract infections were first treated by Hewitt and Wood (1953). None of the 6 patients with chronic urinary infections described by Hewitt and Wood (1953) experienced even symptomatic improvement, although full recovery could not be expected as 5 of them had some obstruction to the passage of urine. The infecting organisms were *Str. faecalis* in 4 patients and *Staph. aureus* in 2 others. Trafton and Lind (1954) were particularly hopeful. In 52 patients with chronic urinary tract infections which were mainly resistant to other antibiotics, carbomycin was given for 7 days in doses amounting to 2 G daily. In spite of the fact that carbomycin was poorly excreted these workers observed that the urine frequently became free of bacteria within 3 days of beginning therapy. The main difficulty appeared to be the presence of enterococci. In patients infected with these organisms, although symptomatic improvement followed carbomycin treatment, the bacteria persisted. When carbomycin treatment was prolonged, however, 40 cases experienced clinical improvement and even those with enterococcal infection remained completely cured 9 months after treatment was first begun, provided the dose of 2 G daily could be maintained. Although the response was slower when enterococci were the main invaders, these organisms did not become resistant to carbomycin. As is usual, *E. coli* was the commonest secondary invader and if this organism became resistant carbomycin was ineffective. The main difficulty in treatment with a daily dose as high as 2 G was diarrhoea and anorexia. Half the patients taking this dose suffered from these complaints and in some patients symptoms were so severe that they necessitated modification or interruption of treatment. In spite of the low levels of the drug in both blood and urine, Trafton and Lind (1954) still claimed that patients responded favourably.

CONCLUSION

The similarity in action between erythromycin and carbomycin calls for careful assessment of the relative value of the 2 antibiotics. The particularly low concentrations found in the blood after practicable oral doses of carbomycin together with the almost exact correspondence in cross resistance between the 2 drugs, leads one to assume that, in common pyogenic infections at least, there is no advantage in using this antibiotic when erythromycin is available.

SPIRAMYCIN

This antibiotic, also known in this country as *Rouamycin*, was first obtained from a soil sample in the north of France. A streptomycetes called *Streptomyces ambofaciens* was the source from which the antibiotic was extracted by Pinnert Sindico, Ninet, Preud'homme, and Cosar (1955). The extract, when purified, was an amorphous base sparingly soluble in water, the sulphate salt was more soluble. Its toxicity was relatively low, the LD₅₀ for mice being 1.5 to 2 G per kg weight by subcutaneous injection and 0.15 to 0.25 G per kg by intravenous injection. Chronic toxicity tests showed that dogs could take 0.5 G per kg daily for 4 weeks without signs of ill effects.

Antibacterial activity

The organisms against which spiramycin was most active *in vitro* were the Gram positive cocci: pneumococci of various kinds, streptococci and staphylococci (Chabbert, 1955). Activity was also found against *N meningitidis*, *N gonorrhoeae*, *C diphtheriae*, and some anaerobes such as *Cl perfringens* (Ravina, Pestel, Eloy, Duchesnay, Albouy, and Rey, 1956a). Some effect against rickettsiae was also claimed (Giroud, 1954). Gram negative organisms were not usually sensitive, but some inhibition of *Klebsiella*, *Aerobacter*, and *Salmonella* was produced. *E coli*, *Pseudomonas*, and *Proteus* were completely resistant (Darbon and Crosnier, 1955). The activity of this antibiotic *in vitro* was less than that of carbomycin or erythromycin, but it was superior to the latter drugs *in vivo*. Table 3 shows the concentrations required to inhibit various bacteria *in vitro*. Resistance could be developed fairly readily *in vitro*.

TABLE 3 ANTIBACTERIAL ACTIVITY *IN VITRO* OF SPIRAMYCIN

| Organism | No. of strains tested | µg per ml required for inhibition |
|------------------------------|-----------------------|-----------------------------------|
| <i>A. aerogenes</i> | 1 | 31 |
| <i>Corynebacterium</i> | 1 | 3 |
| <i>E. coli</i> | 1 | 31 |
| <i>Klebsiella pneumoniae</i> | 1 | 33 |
| <i>Mycobacterium</i> | 1 | 23 |
| <i>N. catarrhalis</i> | 1 | 10 |
| <i>N. gonorrhoeae</i> | | S |
| <i>Proteus vulgaris</i> | 1 | > 1500 |
| <i>Ps. aeruginosa</i> | 2 | 3.9-1,500 |
| <i>Staph. aureus</i> | 18 | 1.4-7.8 |
| | 1 | 31.3 |
| <i>Str. faecalis</i> | 2 | 1-250 |
| <i>Str. pneumoniae</i> | 2 | 0.2-0.24 |
| <i>Str. pyogenes</i> | 4 | 0.12-0.49 |
| <i>Str. viridans</i> | 1 | 1.35 |

S = sensitive

From the data of Ravina, Pestel, Eloy, Duchesnay, Albouy, and Rey (1956a) and Lepper, Spies, Kellow, Rosenthal, and Plant (1956).

Trials *in vivo* showed that mice infected intraperitoneally with virulent haemolytic streptococci, pneumococci, and staphylococci were adequately

protected by the antibiotic. Nodules in rabbits injected intradermally with rickettsial material showed a progressive diminution in size when the animals were treated from the 1st day or later.

Administration

Spiramycin is taken readily by mouth. A dose of 0.75 G given 6 hourly produces mean blood concentrations of 2.7, 2.8, and 1.6 μg per ml at 1, 2, and 6 hours after a single dose in adults. In children given 75 mg per kg of body-weight per 24 hours in a similar way, the serum levels usually varied between 0.06 and 1.0 μg per ml with occasional rises to 4.0–8.0 μg per ml (Lepper, Spies, Kellow, Rosenthal, and Plant, 1956).

Clinical trials

A number of these have been reported from France. Darhon and Crosnier (1955) have treated 17 infections due to Gram positive pathogens with good results. These infections include bacterial endocarditis, enterocolitis, pneumopathies, pansinusitis, and septicaemias of unknown origin, presumably due to Gram positive cocci. These authors gave doses amounting to 2 to 4 G daily for several weeks, until a total of about 100 G had been given. The antibiotic was well tolerated, 1 patient only suffering from a mild gastrointestinal disorder.

Pneumonia has been treated by Renoux, Dupoux, and Huet (1955). Eleven patients with pneumococcal pneumonia recovered satisfactorily under treatment and 7 patients with pneumonia of unknown aetiology which had not responded to penicillin or streptomycin also showed satisfactory results. Again, 18 negroes with pneumococcal pneumonia were treated by Soulagé, Charmot, and Delahousse (1956) with 1.5 to 3 G daily. All of these patients recovered, even 2 with empyema and 1 with pneumococcal nephritis. These results are very encouraging since pneumococcal pneumonia takes a particularly severe form in African negroes. Hudson, Yoshihara, and Kirby (1956) treated a third series of 29 patients with this complaint. These patients were each given 1 G 6 hourly, and 26 of them responded satisfactorily.

Scarlet fever was treated by Martin, Sureau, Chahbert, Veron, Martin, and Cayla (1955) and the results were compared with a parallel series treated with erythromycin. Both antibiotics were found to be equally effective therapeutically but erythromycin was not so well tolerated by adults as spiramycin.

One case of typhus responded satisfactorily to the administration of the antibiotic (Renoux *et al.*, 1955). Ravina *et al.* (1956a) gave the antibiotic to 17 patients with various infections. These patients did not have a good prognosis, being mainly elderly people. The conditions treated were pulmonary congestion, phlebitis, double pneumonia, staphylococcal septicaemia with a renal focus, polymorphous erythema, and chronic osteitis following a compound fracture, and 2 other unspecified conditions in old people of 85 and 89. All of these patients recovered from their infections. Besides these impressive results younger people with acute infections also responded well and promptly to treatment. For example, 1 case with purulent pleurisy due to an unidentified organism cleared up after daily doses of the drug by mouth, amounting to 3 G in 7 days, 12 pleural washouts with 0.25 G of the drug in saline were also carried out. A case of bronchiectasis in a young man

whose sputum contained staphylococci, Gram positive rods, and Gram negative cocci was much improved within 4 days of beginning treatment with the drug by mouth the volume of expectoration fell in 15 days from 150 ml to 25 ml Endocarditis due to an enterococcus which had resisted treatment with oxytetracycline was brought under control Acute infections of the throat responded promptly A condition in which fever and splenomegaly featured cleared up within 15 days Finally, a whitlow of the index finger and 2 cases of furunculosis cleared so readily that, in the former, surgical intervention was unnecessary and in the latter the furuncles subsided in a few days Such satisfactory results were not obtained so uniformly by Lepper, Spies, Kellow, Rosenthal, and Plaut (1956) These workers carried out trials in 124 patients with various infections including acute conditions, and others which were superimposed on chronic disease Divided according to the type of bacterium responsible the case results were summarized as follows

Infections due to β haemolytic streptococci

Forty three cases These included scarlet fever, pharyngitis, and otitis media Of these, 36 responded well

Pneumococcal infections

Thirty one cases Pneumonia was the main condition due to pneumococci, but there were also cases of otitis media and pharyngitis Twenty eight of these responded well but the remainder only fairly or poorly

Staphylococcal infections

Twenty seven cases The results from treatment of these were the most disappointing The conditions treated included soft tissue infections, 7 out of 8 of which responded well otitis media, laryngotracheobronchitis, bronchiectasis pyelonephritis and bacteraemia In the latter condition endocarditis was considered the main focus in 2 cases while in the 3rd multiple liver abscesses were present In contradistinction to the results in the cases of endocarditis treated by Ravina *et al* (1956a), none of the latter patients responded to treatment In all only 13 of the 27 patients were definitely benefited by the treatment Resistance of the infecting organisms was demonstrated *in vivo* in 3 instances but this does not explain the disappointing results in half the cases These may possibly be explained by a fact to which Lepper *et al* (1956) themselves drew attention namely the relatively low serum levels produced by the antibiotic in spite of continued treatment, and the relatively high daily dose necessary These authors also noted that the patients in whom the response was slow or not apparent received the smaller dose of the 2 administered In view of the conflicting results obtained by these groups of workers it is possible to conjecture that the frequency of administration of the drug may have had something to do with the low serum levels found by Lepper *et al* (1956) If the antibiotic is destroyed or excreted rapidly it is obvious that it requires frequent replacement In the early days of penicillin, when supplies were limited, 3 hourly administration was required to keep the serum levels above the concentration required to inhibit the pathogenic organisms being treated and this regime may also be required

with spiramycin. The only untoward effects of the drug in these 124 patients were loose stools in 13 cases. Although there were no serious side effects, it may none the less be advisable to avoid such complications by not raising the daily dose above the 3 G given by Lepper *et al* (1956). One other consequence of importance was the finding by these latter workers that, although 564 cultures of *Staph aureus* obtained from patients and staff in one hospital at the initiation of the study were inhibited mainly by 10 μ g per ml of the drug or less, at a later date a significantly greater number were not inhibited by this concentration. This increase in resistance became more evident in the second 3 months of the study rather than in the first. Moreover, although cross resistance between erythromycin and spiramycin was not common, it did occur (Hudson *et al*, 1956), and its incidence increased as the incidence of erythromycin resistant strains increased. Moreover, strains of staphylococci habituated to one antibiotic become resistant to the other (Chabbert, 1955, Garrod, 1957).

Gonorrhoea

A study of 85 patients with this complaint was undertaken by Willcox (1956 a). Treatment with spiramycin whether in multiple doses over 1 or 2 days or in a single dose of 2 to 4 G was remarkably successful. The most effective regime, however, was that in which the doses were divided and repeated frequently. In a series of 31 treated patients, there was one failure only. Willcox also commented on the lack of significant side effects from treatment with this antibiotic.

Non gonococcal urethritis

Again Willcox (1956 b, 1957 a) made a trial of spiramycin in the treatment of this condition. Eighty seven patients were treated. Although the aetiology of the disease might be in doubt as 60 patients admitted having previously had venereal disease, serological evidence of such was present in 8 only and the gonococcal complement fixation test was positive in only 2. Treatment consisted in administration of tablets of spiramycin 4 times a day for 5 days till a total of 10 G or 20 G had been taken. The results were not so successful as those from the treatment of gonorrhoea, 9 patients failing to respond out of 33 patients given the higher dose and the same number out of 44 given the lower dose. On comparing these results with those obtained from the treatment of non gonococcal urethritis with 10 other antibiotics, only the tetracycline group had proved slightly more successful (Willcox, 1957 a).

Amoebiasis

Bonan, Khouri, Boisoni, and Duponx (1956) treated 14 patients with intestinal amoebiasis in whose stools amoebae were found. The dose they gave was 3 G daily for 3 to 10 days. The patients' response was favourable, their symptoms disappeared in the first few days of treatment, and their stools were free of amoebae after the first treatment. Again the tolerance to the drug was remarked on, no side effects being observed.

OLEANDOMYCIN

First described by Sobin, English, and Celmer (1955), P A 105 or oleandomycin (also known as *Romicil* and *Matromycin*) was found to be a basic antibiotic derived from a strain of *Streptomyces antibioticus*. It was distinguished from other antibiotics by paper chromatography and infra red spectra. When left to stand for 24 hours at room temperature solutions in water were stable, showing no loss of activity at pH 2, 2.5, 7, or 9.

Antibacterial activity

The principal character of oleandomycin is its activity against Gram-positive bacteria, among them being the staphylococcus, but it also inhibits mycobacteria, rickettsiae, large viruses, and certain protozoa. Except for *Neisseria*, *Haemophilus*, and *Brucella*, it is not active against Gram negative bacteria.

Table 4 gives a list of bacteria and the concentrations which inhibited them *in vitro*.

TABLE 4 ACTIVITY *IN VITRO* OF OLEANDOMYCIN

| Micro organism | Minimal inhibiting concentration ($\mu\text{g/ml}$) |
|--|---|
| <i>M. pyogenes</i> var. <i>aureus</i> * | < 0.19-22.5 |
| " <i>albus</i> | 0.78-2.5 |
| <i>Str. faecalis</i> | 0.312-4.88 |
| <i>Str. pyogenes</i> | 0.078 |
| Non haemolytic streptococci | < 0.09-3 |
| <i>Str. viridans</i> | < 0.156-1.25 |
| <i>Bacillus subtilis</i> | 0.39 |
| <i>Bacillus anthracis</i> | 0.78 |
| <i>Bacillus mycoides</i> | 1.56 |
| <i>Erysipelothrix rhusiopathiae</i> | < 0.19-0.39 |
| <i>Dipl. pneumoniae</i> | < 0.07-30 |
| <i>C. diphtheriae</i> | 1.56 |
| <i>Listeria monocytogenes</i> | 0.78 |
| <i>Cl. tetani</i> | 8.25 |
| <i>Cl. sporogenes</i> | 3.12 |
| <i>Mycobacteria phlei</i> and <i>smegma</i> | 1.56 |
| <i>Mycobacterium tuberculosis</i> H37 Rv | < 100 |
| " (bovine) | 12.25-< 100 |
| <i>Brucella bronchiseptica</i> | 6.25 |
| <i>Neisseria catarrhalis</i> | 3.12 |
| <i>Neisseria meningitidis</i> | 1.56 |
| <i>Neisseria gonorrhoeae</i> | 3.12 |
| <i>Haemophilus influenzae</i> | 0.078 |
| Other Gram negative bacteria and <i>Candida albicans</i> | > 100 |

After Sobin *et al* (1955) Garrod (1957) Fust, Bohm, Zbinden, and Studer (1958), and Hobby, Celmer, Lenert, Pikula, Vrabeč, Donikian, Dely, and Sarroco (1957).

* *Staphylococcus aureus* in English texts.

Resistance

Comparison of the minimal concentrations of oleandomycin required to inhibit 21 different strains of *Staph aureus* with those required by penicillin, streptomycin, the tetracyclines, chloramphenicol, polymyxin B, or bacitracin showed that there was no cross resistance, but one strain which had developed resistance to carhomycin and erythromycin in the laboratory was also found to be resistant to oleandomycin. Work on enterococci and staphylococci isolated from urinary tract infections by Trafton and Land (1957 *a* and *b*) also demonstrated cross resistance to the 2 antibiotics. Comparison of the susceptibility *in vitro* of the organisms they isolated to erythromycin and oleandomycin showed that whereas those that were sensitive to erythromycin were also inhibited by oleandomycin, the reverse did not hold good. Strains of either species which were resistant to erythromycin were not always so to oleandomycin. Cross resistance therefore was not complete.

There was good therapeutic activity against experimental infections in mice with *Str pyogenes* and *Staph aureus*. All animals survived for 96 hours after intraperitoneal inoculations with lethal doses and subcutaneous injections of 30 to 60 mg per kg of the drug begun half an hour after infection.

The toxicity of oleandomycin was not great as judged by experiments on mice. The LD₅₀ for these animals was 550 mg per kg body weight when given intravenously. Later work on rats, dogs, and monkeys by Sorensen, Fiske, Reutner, Weston, and Weston (1957) showed that though the acutely toxic dose produced by intravenous injection in rats was about half as high as that for mice, chronic toxicity tests in dogs revealed that these animals could tolerate oral administration of as much as 300 mg per kg of weight daily for 5 days a week over 40 to 41 weeks. Monkeys on a similar regime, however, were not free from some disturbance of the blood cell counts and one animal, on death, showed a marked degree of nephritis.

Because of the cross resistance induced in strains of *Staph aureus* to erythromycin and oleandomycin confirmed by Needham and Geraci (1956) and Noyes, Nagle, Sanford and Robbins (1956), there was delay in making this antibiotic available to the medical profession. However, when English, McBride, van Halsema, and Cariozzi (1956) had demonstrated synergism between oleandomycin and tetracycline against selected organisms and the ability of the combination to retard the emergence of resistance in certain strains of *Staph aureus* and *Str pyogenes*, the combination was made available as *Sigamycin*. Unfortunately Garrod (1957) could not confirm the synergistic activity of these 2 antibiotics against *Staph aureus*, nor could Fairbrother and Southall (1957) do so often or to any extent. This does not necessarily mean that some delay in the appearance of resistant strains may not be accomplished by use of the 2 drugs together.

Administration

Oral administration having been found to be practicable in animals (Kazenko, Sorensen, Wolf, Dill, Galbraith, and Glazko (1957) Baad, Corbin, and Prigot (1957) assayed the concentrations to be found after oral administration in man. A dose of 500 mg was given every 4 hours from 8 a.m. to 8 p.m. the next day to 50 male patients or a 1 G dose was given at 8 a.m. and 8 p.m. The concentrations these workers found are given on p. 52.

µg. per ml in the blood serum at different times during administration of oleandomycin by mouth

| Dose | 8 a.m. | 9 a.m. | 12 noon | 4 p.m. | 5 p.m. | 1 st mid night | 8 a.m. | 9 a.m. | 12 noon |
|-------------------------------------|--------|--------|----------------|---------------|---------|---------------------------|--------|---------|---------|
| 500 mg 4 hourly 8 a.m. to 8 p.m. | 0* | 0-1 25 | 0-0 6 | 0-1 25 | 0-1 25 | 0-2 5 | 0-5 0 | 0-0 6 | 0-1 25 |
| Average | | 0 515 | 0 292 | 0 768 | 0 577 | 1 268 | 1 599 | 0 275 | 0 768 |
| 1 G at 8 a.m. and 8 p.m. | 0* | 0-0 6 | 0 156- 1 25 | 0 31- 2 15 | 0-0 625 | 0-1 75 | 0-1 03 | 0-0 312 | 0-2 5 |
| Average | | 0 279 | 0 74 | 1 810 | 0 312 | 0 514 | 0 331 | 0 187 | 1 45 |

* Control assays made before injection of oleandomycin

These assays do not tell us very much except that with doses planned according to these two schemes it is obvious that bacteria would have to be inhibited by 1 µg per ml or less in order to be controlled by oleandomycin

Clinical trials

When given alone oleandomycin yet produced satisfactory results in a number of infections. Bunker and Schutze (1956) described its use in 90 patients suffering from suppurative processes caused by staphylococci, streptococci, enterococci or pneumococci. Oleandomycin was administered as tablets or ampoules 4 or 8 hourly in an average daily dose of 33 mg per kg of body weight. This usually amounted to 500 mg given 4 times a day. A few patients were given the drug intravenously or intramuscularly as 1 G per 24 hours over 8 to 10 days. Tolerance to the drug was good and the therapeutic results were excellent in 58 patients with pneumonia, pulmonary abscesses, empyema, furunculosis, sore throat, staphylococcal infection of the skin, endocarditis caused by micrococci,¹ parotitis, urinary tract infections, and ornithosis.

Bunker and Schutze carried out sensitivity tests on primarily isolated bacteria and found 95 per cent of them, though resistant to penicillin, streptomycin, or tetracycline, were sensitive to oleandomycin. Three strains which were resistant to erythromycin and penicillin were also resistant to oleandomycin. These authors also stated that acquired resistance to oleandomycin was slow. A higher dose was chosen by Siegenthaler, Keiser, and Hegglin (1956) who gave their adult patients 1.5 to 2 G 6 hourly and 40 mg per kg body weight daily to children. There were few if any side effects in their 216 patients, 1 instance of enterocolitis not being with certainty ascribed to oleandomycin. These workers considered its action to be similar to that of erythromycin; they also found instances of primary and acquired resistance among the infections treated. There were cases of atypical pneumonia, Q fever, and leptospirosis which Siegenthaler *et al* thought were favourably affected and 6 carriers of *C. diphtheriae* were cleared of their carrier state. These workers concluded that oleandomycin had been a useful therapeutic agent but thought it should be given with another drug to prevent resistant infection from developing. Another enthusiastic report was that of Essellier and Keith (1956) who treated 412 cases with the antibiotic. They found the results were particularly good in upper respiratory tract infections, in pneumonia and pulmonary abscess, also in various soft tissue infections.

¹ Usually described as staphylococci in English texts

and osteomyelitis (generally supposed to be staphylococcal in origin), and a number of other inflammatory conditions including urinary tract infections due to Gram positive organisms. What is more surprising is that they reported good results from the treatment of Q fever and virus pneumonia.

Diphtheria

A trial of oleandomycin in diphtheria and diphtheria carriers was carried out by Gagliardi (1956) who treated 73 patients, mainly children. Seventeen of these had pharyngeal diphtheria, 2 nasal diphtheria, and 54 were carriers. The children were given 100 mg. as a pill every 3 to 4 hours for 5 to 6 days. There were no untoward side effects and *C. diphtheriae* disappeared from the nose and pharynx in about half the time that is usually expected. Carriers were free within 16 days in 90 per cent. of the patients instead of taking 26 to 40 days to lose the pathogen. As the cases of diphtheria were mild, Gagliardi did not feel justified in pronouncing on the clinical effects of the treatment.

Gonorrhoea

Seventy one male patients with acute gonococcal urethritis were treated by Marmell and Prigot (1957 a). In order to find the most satisfactory scheme of dosage, Marmell and Prigot divided the patients into 3 groups, the first receiving 400 mg. in gelatin capsules 3 times a day for 2 consecutive days, the second the same dose 4 times a day over the same time, and the third 1 G. to start with followed by 500 mg. 6 hourly for 5 doses. With a criterion of cure which postulated that the urethral discharge should have disappeared and smears and cultures be negative for 3 consecutive days following treatment, their results were as follows:

| Dosage | Total patients | Total observed* | No. cured | No. failed |
|--------|----------------|-----------------|-----------|------------|
| 1st | 13 | 11 | 7 | 4 |
| 2nd | 41 | 30 | 25 | 5 |
| 3rd | 17 | 10 | 9 | 1 |

* Remainder failed to report for the full period of observation

From these results it would appear that, provided patients are willing to submit to treatment for 2 days, their acute attacks of gonorrhoea should be cured with a course of 1 G. followed by 500 mg. 6 hourly.

Granuloma inguinale

Three patients with lesions about the anus or labia minora were treated by Marmell and Prigot (1956 a). The diagnosis was confirmed by finding Donovan bodies in smears and then having negative Frei and Ito Reinstierna tests. They received 250 mg. by mouth 4 times a day till healing had taken place. While admitting that the rate of healing depended on the extent of the lesion, Marmell and Prigot were able to observe that each lesion did heal on total doses of 25, 30 and 76 G. respectively in spite of their being present for periods of up to 5 years before treatment.

Urinary tract infections

Trafton and Lind (1957 b) having carefully selected patients with staphylococcal and enterococcal infections which were not resistant to oleandomycin,

ie to 125 μ g per ml, treated 14 cases with 1 to 2 G daily for 10 days to 3 weeks. Describing 'cure' as elimination of pyuria or sterilization of urine and 'improvement' as marked decrease in pyuria, these workers found that 6 patients were cured and 5 improved. As the majority of them were suffering from chronic disease or had some obstruction to the flow of urine which had required operative measures, the results appear remarkably good and support the contention that for an antibiotic to have an effect, the responsible organism must be susceptible. Yet this is not the only factor necessary for recovery.

Clinical trials of combinations with oleandomycin

The experimental basis on which therapy by combinations with oleandomycin was built began with the work of English *et al* (1956), by which they found greater activity against various organisms than was possessed by either drug separately when oleandomycin was combined with tetracycline in the proportion of 1:2. Other investigators observed the effect on the inhibitory activity of the plasma in patients receiving oleandomycin and penicillin. Jones and Finland (1957*b*) compared the height of blood levels after administering by mouth equivalent doses of the 2 antibiotics together and separately. Using as their test organisms *Str. haemolyticus* 98, *Staph. pyogenes* 209 P, or *Staph. pyogenes* 2351, they found that the inhibitory concentration rose to a distinctly higher level after administration of combinations of the 2 antibiotics than after oleandomycin given alone, but that the oleandomycin constituent of the combination ensured maintenance of inhibitory concentrations for a slightly more prolonged period than did penicillin alone. Comparing the activity *in vitro* of the salt oleandopen, made up of 2 parts of oleandomycin to 1 part of penicillin, with that of penicillin G and of oleandomycin, Hobby, Celmer, Lenert, Pikula, Vrabec, Donikian, Daly, and Sarroco (1957) found that the salt inhibited strains of *Str. haemolyticus*, *Dipl. pneumoniae*, and enterococcus in concentrations as low as those required with the more active antibiotic alone, but with certain strains of *Staph. aureus* this concentration was lower than that required by the more active antibiotic separately. When assaying the inhibitory activity found in the serum of man after oral administration of oleandopen, Payne, Hobby, Thornhill, Terry, Hackney, Spurlock, Syphax, Daly, Sarroco, and MacNeil (1957) concluded that the salt was readily absorbed and that it produced initially high levels maintaining inhibitory concentrations over 8 hours. Addition of penicillin G to the combination raised the initial concentrations without having much effect on their maintenance. From their assays they recommended a dose of 500 mg of oleandopen every 8 hours as sufficient to maintain inhibitory levels. These studies appear to show that the salt oleandopen may be useful for certain staphylococcal infections where sensitivity tests have indicated that it is more active than oleandomycin or penicillin alone. Clinical trials in 7 patients indicated its therapeutic effectiveness without comparison with the outcome in similar conditions treated with oleandomycin or penicillin alone. The salt was not free from producing side-effects in the form of diarrhoea.

Other trials have been made with combinations of oleandomycin and one of the tetracyclines, that with oxytetracycline was described as PA 765 and that with tetracycline as PA 775 (known also as *Sigmamycin*). In each the

proportions were 2 of the tetracycline to 1 of oleandomycin. Acute and chronic toxicity in animals having been shown to be of a low order (Kaiser, Mazzarino, Bajek, and P An, 1957), the way was open for clinical application. Though no enhancement of activity in the plasma was observed against a

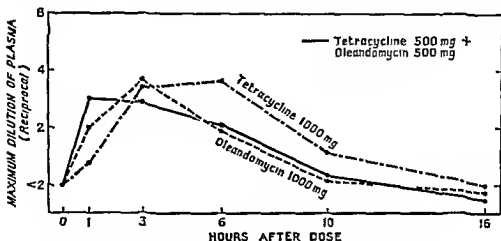


FIG 2a Average levels of inhibition of *Staph 209P* by plasma after ingestion of Tetracycline 1000 mg ————
Oleandomycin 1000 mg - - - - -
or tetracycline and oleandomycin 500 mg of each respectively ————

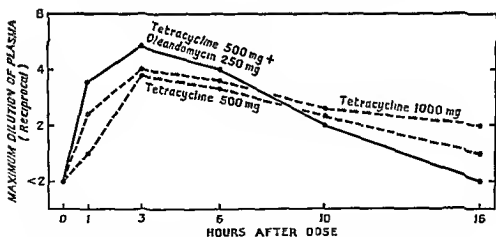


FIG 2b Composite figure showing average levels of inhibition of *Str 98* after ingestion of doses of 1000 mg each of tetracycline or oleandomycin or of 500 mg tetracycline together with 500 mg of oleandomycin

sensitivity *in vitro* of *Str 98* to tetracycline— $2.3 \pm 1.5 \mu\text{g}$ per ml

sensitivity *in vitro* of *Str 98* to oleandomycin— $3.8 \pm 0.6 \mu\text{g}$ per ml

(From Jones and Finland *New Engl J Med* 1957 *h* 257, 536)

staphylococcus or a streptococcus by Jones and Finland (1957 *h*) when doses of each of the antibiotics were given, in conjunction or their equivalents separately (Fig 2 a and b), there should be some hope of dealing with mixed infections and of delaying the emergence of resistance in an infection which was initially susceptible. Such results, however, do not seem to have been observed in clinical trials.

Gonorrhoea and lymphogranuloma venereum

The only series of cases of specific infection which were treated seem to be those of Marmell and Prigot (1957 *a* and *b*). They treated both conditions with the oleandomycin oxytetracycline combination. To 30 male patients with acute gonorrhoea were administered either 500 mg to start with, followed by 250 mg 6 hourly for 2 doses, or 500 mg 3 times in 1 day. After the first treatment there were 5 failures among 20 patients and, after the second, 1 failure among 10. Comparison of these results with those obtained by these workers using oleandomycin or oxytetracycline alone (Wright, Metzger, Beinfield, Di Lorenzo, and Marmell, 1951 *a*) shows that little if anything was gained by using the combination. Three patients with lymphogranuloma venereum were also treated with the combination, this time by only 250 mg given 4 times a day for 15 to 20 days. Under this treatment all lesions healed. Such a result could hardly be bettered without carefully controlled trials but they were certainly equalled by oxytetracycline in the hands of these same workers and their colleagues (Wright *et al.*, 1951 *a*; Wright, Parker, Allen, and Beinfield, 1951 *b*).

Miscellaneous clinical trials

Two hundred and fifty six patients, both children and adults, suffering from conditions not considered to endanger life were treated with one or other of the combinations by Carter and Maley (1957). The responsible organisms were considered to be *Staph aureus*, *Staph albus*, α streptococci, *Str faecalis*, pneumococci, *Klebs pneumoniae*,¹ *A aerogenes*, and *Proteus* species, most of which were resistant to one or more of the following antibiotics: penicillin, tetracycline, erythromycin, and chloramphenicol. Following treatment with 1 or 2 capsules each containing 250 or 300 mg of 'total antibiotic activity', 4 times a day, the patients recovered from their various infective conditions which included pharyngitis, bronchitis, bronchopneumonia, lobar pneumonia, osteomyelitis, cellulitis, pyogenic arthritis, and gastro enteritis, their temperatures being normal in 1 to 4 days. There was 1 failure, however, in a patient with osteomyelitis due to an unidentified organism and 1 death in a patient with an herpetic infection and suspected bronchopneumonia. At autopsy this patient was found to have extensive herpetic lesions of the trachea and bronchi, a condition which could not be expected to benefit from these antibiotics. There was no gastro intestinal intolerance or evidence that the elements of the blood had been affected. One skin eruption appeared 3 days after treatment had been stopped. Shubin (1957) also made a trial with the tetracycline-oleandomycin combination in a miscellaneous group of infections. He treated 167 patients whose infections were due to organisms insensitive to the main antibiotics and 40 in whom the organisms were sensitive to them. With a dose of 500 mg administered 3 hourly to start with but with gradually increasing intervals between doses he found the results encouraging and in some patients he considered the effect of the drug had been life saving.

Other trials in the treatment of bacterial diseases were made by La Caille and Prigot (1957) and Winton and Chesrow (1957), and of tropical infections by Loughlin and Mullin (1957). From their reports one concludes that

¹ Usually known as *Bacillus friedlanderi* in English texts.

either combination, in doses of approximately 300 mg every 6 hours, had a favourable influence on soft tissue infections presumably due to Gram positive bacteria, causing inflammation to subside, and limiting the number of cases requiring incision and drainage as well as the extent of the incisions. In tropical infections both combinations were found capable of ridding the faeces of *E. histolytica*, in both cyst passers and patients with symptoms of amoebiasis of ensuring healing of yaws, lymphopathia venereum, and tropical ulcer. These latter two conditions were treated topically as well as by mouth. All the patients with tropical diseases were treated with higher single doses than those used for bacterial infections, usually being given 3 to 6 capsules (600–1,200 mg of the tetracycline and 300–600 mg of oleandomycin) once daily, but healing progressed in spite of the relatively long intervals between administration. No toxic reactions of significance were observed except a mild stinging from local application (Loughlin and Mullin, 1957).

Diseases of the skin—acne

Cornbleet and Firestein (1957) attempted to assess the effect of oleandomycin and tetracycline (*Sigmamycin*) on 26 cases of acne. Treatment was necessarily prolonged, leading these investigators to make their assessments after 2 weeks' and after 8 weeks' treatment. Four capsules were given each day or, sometimes when response was not apparent, the dose was increased to 8 capsules. With increase in the dose gastro-intestinal upsets were observed such as bloating with much flatus or diarrhoea. Nevertheless in those patients who persisted in their treatment improvement was usually evident. The results were as follows:

| Duration of treatment | No of patients | Good | Fair | Results | |
|-----------------------|----------------|------|------|---------|------|
| | | | | | Poor |
| 2 weeks | 26 | 5 | 16 | | 5 |
| 8 weeks | 19 | 9 | 8 | | 2 |

No note was made of accompanying pyogenic infection or of any bacteriological examinations. There is thus some reason to wonder whether the improvement noted would have occurred with the passage of time and without the help of antibiotics.

Conclusion

From the results of clinical trials it would seem legitimate to conclude that oleandomycin has proved effective in acute infections caused by Gram-positive bacteria. There is some doubt as to its ability to control chronic infections where treatment must be prolonged. Because of the risk of cross resistance manifested by some staphylococci to it and to erythromycin, it would seem advisable to limit the use of oleandomycin to patients or institutes where erythromycin is not employed.

CHAPTER 3

ANTIBIOTICS WHICH CONTROL THE STAPHYLOCOCCUS AND OTHER GRAM-POSITIVE BACTERIA

NOVOBIOCIN, VANCOMYCIN, RISTOCETIN, STAPHILOMYCIN,
AMPHOMYCIN

NOVOBIOCIN

GENERAL CONSIDERATIONS

THIS antibiotic was prepared separately in the laboratories of the Upjohn Co., of the Chemical Division of Merck & Co., Inc., and of Chas Pfizer & Co. It was originally thought to be a number of different antibiotics, which were given different names: streptomycin or *Albamycin*, *Cathomycin*, and *Cardelmycin*. The resulting confusion was eventually reduced when it was found that all these compounds had the same composition and activity (Hoeksema, Bergy, Jackson, Shell, Hinman, Fonken, Boyack, Caron, Ford, Devries, and Crum, 1956; Jones, Nichols, and Finland, 1956; Welch and Wright, 1955). The name 'novobiocin' was given to the antibiotic by the American Food and Drug Administration (1956).

Novobiocin was obtained from a newly discovered species of streptomyces called *Streptomyces spheroides* or *niveus*, which grew in an old sod pasture in Vermont, U.S.A. (Wallick, Harris, Reagan, Ruger, and Woodruff, 1956). According to these workers it was isolated in crystalline form by Kazka, Rathe, and Folkers. Unlike erythromycin and its allies it is weakly acid in reaction, the sodium salt is soluble in water, but the calcium salt is more stable in solution (Council on Drugs, 1957).

Bacteriological properties

Studies of its antibacterial action by Wallick *et al* (1956) indicated that it had some activity against *Staph aureus*, *Str pneumoniae*, *Str pyogenes*, and *Str faecalis* and also against the Gram negative rods, *Shigella* and *E coli*. Verwey, Miller, and West (1956) carried out more detailed studies using an amorphous mono sodium salt of the antibiotic. These tests, together with others made with the crystalline material form the basis of Table 5 given below.

From Table 5 it is seen that staphylococci are most uniformly susceptible to the antibiotic. The Gram negative rods, except for the haemophili, are the least susceptible. Streptococci vary considerably in their sensitivity. The uniform susceptibility of staphylococcal strains to novobiocin was confirmed by disk plate methods by both Rantze, Randall, Thum, and Barker (1957) whose 41 strains isolated from different patients were all inhibited by 1 to

10 μg per ml, and by Fairbrother and Williams (1956) whose 470 strains freshly isolated from infective processes were all inhibited by 10 μg per ml

TABLE 5 ANTIBACTERIAL ACTIVITY OF NOVOBIOCIN *IN VITRO* AGAINST ORGANISMS PATHOGENIC TO MAN

| Species | No of strains tested | μg per ml required for inhibition |
|--|----------------------|--|
| <i>A. aerogenes</i> | 17 | 50-> 400 |
| <i>Br. abortus</i> | 1 | 31.2 |
| <i>C. diphtheriae</i> | 6 | 0.2-0.78 |
| <i>E. coli</i> | 14 | 100-400 |
| <i>E. rhusopathiae</i> | 1 | 25 |
| <i>H. pertussis</i> | 1 | 0.06 |
| <i>H. influenzae</i> | 5 | 0.4-0.8 |
| <i>Klebsiella pneumoniae</i> | 3 | 18.8-> 200 |
| <i>Myc. tuberculosis</i> | 1 | 31-62 |
| <i>N. meningitidis</i> | 8 | 0.39-1.6 |
| <i>N. gonorrhoeae</i> | 3 | 0.4-6.3 |
| <i>Pasteurella species</i> | 14 | 1.6-12.5 |
| <i>P. vulgaris</i> | 52 | 1.5-> 100 |
| <i>P. mirabilis</i> | 10 | 25-100 |
| <i>P. morгани</i> | 6 | 50-100 |
| <i>P. rettgeri</i> | 3 | 50-400 |
| Other proteus | 18 | 1-400 |
| <i>Ps. aeruginosa</i> | 9 | 50-500 or more |
| <i>Salm. typhosa</i> and other salmonellae | 23 | 12.5-> 400 |
| <i>Shigella sonnei</i> and <i>shigae</i> | 6 | 38-> 400 |
| <i>Staph. aureus</i> | 137 | 0.05-> 50* |
| <i>Str. pyogenes</i> | 40 | 0.2-100 |
| <i>Str. pneumoniae</i> | 32 | 0.2-12.5 |
| <i>Str. faecalis</i> | 22 | 3.1-25 |
| <i>Str. viridans</i> | 14 | 3.1-100 |

* Majority inhibited by 0.39 to 1.56 μg per ml

From the data of Lin and Cornell (1956), Frost, Valiant, McClelland, Solotorovsky, and Cuckler (1956), Lubash, Van der Meulen, Bernstein, and Tompsett (1956), Martin, Heilman, Nichols, Wellman, and Geraci (1955), Jones, Nichols, and Finland (1956), Simon, McCune, Dineen, and Rogers (1956), Verwey, Miller, and West (1956), Wallick, Harris, Reagan, Ruger, and Woodruff (1956)

Agents affecting the activity of novobiocin

Using a staphylococcus as the test organism, Jones *et al* (1956) found that defibrinated horse blood or serum decreased the activity of the antibiotic. The inhibitory effect of serum increased with concentration, and the bactericidal action of the drug was also interfered with. These findings were confirmed by Martin *et al* (1955), Kirby, Hudson, and Noyes (1956), Lubash *et al* (1956), and Simon *et al* (1956), and were extended to other organisms such as the enterococcus. Bovine albumin was found to interfere as well as serum. Lowering the pH of the broth medium from 8.1 to 5.4 improved the antibacterial action but, according to Lubash *et al* (1956), not significantly so. The size of the inoculum also affected the activity of novobiocin, the heavier the inoculum the less active the antibiotic. The action of novobiocin was found to be bacteriostatic rather than bactericidal except in concentrations many times greater than those inhibiting the growth of the staphylococcus (Lin and Cornell, 1956).

Acquired resistance

Unfortunately resistance could be induced *in vitro* quite rapidly, all strains of staphylococci studied by Jones *et al* (1956) becoming resistant to 400 μg per ml after as little as 7 to 20 subcultures. Lubash *et al* (1956) succeeded in producing 125 fold resistance in 1 strain over 12 transfers. Pneumococci also developed resistance, but more slowly. No cross resistance could be demonstrated between novobiocin and penicillin, streptomycin, oxytetracycline, chlortetracycline, chloramphenicol, erythromycin, or cycloserine (Bayne, Strickland, Gylfe, and Boger, 1956, Coppo, 1957, Martin *et al*, 1955). It was this absence of cross resistance which distinguished novobiocin from the erythromycin group of antistaphylococcal antibiotics described in preceding pages. With vancomycin (p 71) a certain degree of cross resistance was observed by Schneerson and Amsterdam (1957). A curious feature noted by Lin and Coriell (1956) was that some of the strains originally resistant to the commonly used antibiotics became sensitive to penicillin again once resistance to novobiocin was induced. This occurred in 4 strains out of 10 tested, but may be coincidental. This finding still awaits confirmation.

Effect on experimental infections in animals

As soon as it became available novobiocin was compared with other antibiotics in experimentally induced infections in animals. In mice, it compared favourably with erythromycin in acute and chronic staphylococcal infections. Although the majority of animals survived, staphylococci were not, however, eliminated from the tissues (Martin *et al*, 1956a). Novobiocin was less effective than erythromycin in streptococcal and pneumococcal infections. Novobiocin was also found to protect mice from infections with *Past multocida*, *Proteus vulgaris*, and *H influenzae* (Bayne *et al*, 1956, Simon *et al*, 1956). It had no therapeutic activity against experimental infections with tubercle, fungi, rickettsiae, or viruses (Frost *et al*, 1956), nor against *Ps aeruginosa* or *S typhi* infections (Bayne *et al*, 1956).

Administration

Administration by mouth

Concentrations in the blood have been determined after doses of the drug given by mouth. According to Lin and Coriell (1956) these are relatively high in children compared with the levels attained by other antibiotics with similar doses, and many times higher than the concentration required to inhibit sensitive staphylococci. The levels following different single doses are given below.

| Dose (in mg per kg body weight) | No of cases | Diseases | 1 | μg per ml at hours after dose | 2 | 4 | 6-8 | 7-8 |
|--|----------------|------------------------------------|---------|--|--------|---------|-------|-----|
| 5 | 3 | Tetanus | . | 54->64 | 51-60 | 13.5-32 | 7-25 | |
| | | Acute poliomyelitis | | | | | | |
| 10 | 11 | Meningitis (<i>H influenzae</i>) | 24->100 | 5->64 | <4->64 | 9.9-33 | <4-54 | |
| | | Poliomyelitis (4) | | | | | | |
| | | Convulsions | | | | | | |
| | | Headache | | | | | | |
| | | None (4) | | | | | | |
| 20 | 3 | Tetanus | 32-96 | 28->64 | 17->64 | 14-32 | | |
| | | Poliomyelitis | | | | | | |
| | | Meningitis (<i>H influenzae</i>) | | | | | | |

In adults concentrations were also found to reach high levels

| Dose | No of subjects | μg per ml in plasma at hours after dose | | | | |
|--------|----------------|--|---------|-----------|---------|---------|
| | | 0 | 1 | 2 | 4 | 6 |
| 0.25 G | 7 | | | 6.32-11.4 | 2.4-3.3 | 7.24-6 |
| | 6 | <3 | 8 | 10 | 8 | <3 |
| 0.5 G | | | 0.5-1.2 | 6.4-8.4 | 12-50 | 1.6-100 |
| | | <3 | 18 | 25 | 20 | 10 |
| 1.0 G | | | 25 | 35 | 50 | 40 |
| | | <3 | | | | |

From the data of Bayne *et al* (1956), Lubash *et al* (1956), Martin *et al* (1955), and Simon *et al* (1958)

Average values in italics

Much higher average values were obtained by Nichols and Finland (1956) 24 hours after 6 hourly treatment commenced after 0.25 G, 66 μg , after 0.5 G, 76 μg , after 0.5 G, 8 hourly, 33.4 μg , and after 1 G 6 hourly, 48 μg per ml

When doses were repeated it was found that concentrations increased over 5 days. It was therefore considered advisable to limit the frequency of administration to 6 hourly with low doses of 0.25 G, and 12 hourly as doses increased to 0.5 G (Council on Drugs, 1957)

Intramuscular administration

This method has been found useful at times when patients refuse or are unable to swallow the antibiotic. In 7 patients given 0.25 G by injection the concentrations found in the serum by Lubash *et al* (1956) were as follows

| Amount injected | μg per ml at hours after dose | | | |
|-----------------|--|----------|---------|-------|
| | 1 | 2 | 4 | 6 |
| 0.25 G | 3.2-12.8 | 3.2-12.8 | 1.6-6.4 | 0-1.6 |

Till the report of David, McCawley, and Dehult (1957) this form of administration was painful and therefore to be avoided if possible

Distribution in body fluids and tissues

Assays carried out by Lubash *et al* (1956), Martin *et al* (1955) and Simon *et al* (1956) after at least four 6 hourly doses of 0.5 G by mouth showed that, with the exception of the cerebrospinal fluid of patients without inflamed meninges, most body fluids contained novobiocin in easily detectable concentrations. This applied to ascitic fluid, pleural fluid, bile, and urine. In ascitic and pleural fluids the concentrations were lower, but in bile and urine they were higher than in the serum. However, only a fraction of the dose given appeared in the urine during the first 24 hours after ingestion of 0.5 G of novobiocin.

In the faeces, 12 hours after 6 hourly doses had been given for 24 hours, novobiocin appeared in greater concentration than in the serum. In thyroid tissue the amount found varied from none at all to one quarter of the concentration found in the serum. In dogs treated for 60 days the distribution in tissues was found to be widespread, being highest in the bile. Much lower concentrations were found in the liver, lungs, serum, kidneys, spleen, and brain, in that order. None was found in the cerebrospinal fluid (Larson, Connor, Swoap, Runnels, Prestrud, Eble, Freyburger, Veldkamp, and Taylor, 1956)

Toxicity

Toxicity tests carried out on laboratory animals by Larson *et al* (1956) showed a moderate toxicity for mice and guinea pigs in acute tests. Short-term intravenous administration to dogs produced changes in the liver and kidneys, as well as transient *haemoglobinaemia* and irritation of the veins through which the transfusion was being given. Chronic toxicity studies in rats and dogs, however, produced no significant changes in the organs, and in cats subcutaneous administration did not affect the 8th cranial nerve nor have any demonstrable systemic ill effect. Repeated injections over 70 days caused considerable local injury to muscle, but no other toxic effect was produced. In man Martin *et al* (1955) in many trials found no damage to kidneys, liver, or haemopoietic system. Some patients, however, complained of nausea, but no further ill effects were noted. When treatment was continued for a week or more, fever or pruritic rashes occurred occasionally in the patients described by Jones, Nichols, and Finland (1956). A rash also developed in one of the 6 patients described by David and Burgner (1956). To assess the incidence of the reactions Welch, Lewis, Putnam, and Randall (1956) carried out trials on 208 male subjects by giving them novobiocin by mouth twice a day in doses of 0.5 G over a 12 day period. One of these exhibited a maculopapular rash on the arms and chest, while 3 subjects showed a yellow discoloration of the sclera. Only the patient with the skin rash developed a positive reaction when tested by a cutaneous test. Other patients to the extent of 37 per cent had loose stools but not severely enough to necessitate stopping the antibiotic. In some of the 22 patients treated by David and Burgner (1956) mild neutropenia was also observed occasionally as well as eosinophilia and thrombocytopenic purpura developed in a child treated for 10 days by Breese, Disney, and Talpey (1957). Liver function tests carried out by these workers showed an increased icteric index or an elevated indirect van den Bergh test in half the patients tested. It is possible that these findings were due to a yellow pigment derived from novobiocin itself. It is said that this pigment gives an increased total bilirubin reading, but not a direct van den Bergh reaction (Gibson, personal communication to David and Burgner, 1956). Slight increases in the indirect van den Bergh reaction could therefore be attributed to the pigment in the plasma. However, a report of a patient receiving 2 G 12 hourly who developed jaundice and a purpuric rash on the 6th day of treatment, death following 5 days later, leads one to be chary of interpreting elevated van den Bergh reactions in this way. At necropsy Bridges, Berendes and Good (1957) found generalized lymph node hyperplasia and acute diffuse hepatic necrosis, both of which they attributed to the action of novobiocin.

Clinical trials

These have been chiefly reported in *Antibiotic Medicine* (1956, 2, No. 4). The earliest trials seem to have been carried out by Lin and Coriell (1956) at the Children's Hospital of Philadelphia. These were made on 6 children with staphylococcal or streptococcal infections. With 6 hourly doses amounting to 6-10 mg per kg of body weight per 24 hours most encouraging results were obtained: the organisms were reduced in number or disappeared, and the symptoms subsided promptly. In well over 600 cases treated by

different workers¹ and selected because they were unresponsive to other commonly used antibiotics it was generally agreed that acute staphylococcal infections responded well to treatment. Other infections which responded were pneumococcal pneumonia, cutaneous anthrax (1 case), and a certain proportion of infections due to proteus organisms. amoebiasis was controlled at least temporarily in approximately half the cases treated, irrespective of whether the patients were only cyst passers or whether they suffered from ulceration and diarrhoea. Reactions to the drug were limited to urticarial dermatitis which, however, was by no means infrequent. Evidence was adduced by McHardy *et al* (1956) that the side effects produced by 2 G daily were no worse than when the dose was increased to twice that amount. Most reports are particularly optimistic, but the work of Nichols and Finland (1956), who brought a particularly critical attitude to their trials, indicated that there were a number of pitfalls for those who used this antibiotic. In the 52 patients studied by these authors there was evidence that some of the infecting organisms acquired a degree of resistance which resulted in their not being amenable to treatment. Of the 22 staphylococcal infections treated, the sensitivity of the bacterium isolated was tested both before and after treatment in 10 of the cases and in 4 of these resistance had increased 16 to 250 fold. In those lesions where the staphylococcus was inhibited by 31 μg per ml or less good progress was made and even in those where the inhibitory concentration had reached 12.5 μg per ml improvement occurred in lesions which were not deep seated. Infections with proteus organisms or *E. coli* were liable to behave in the same way and with these organisms resistance on occasion rose to 400 μg or more per ml.

Taking the trials as a whole the results of treating various conditions mainly due to staphylococci are summed up in the following paragraphs.

THE TREATMENT OF DISEASES DUE TO SPECIFIC ORGANISMS

Staphylococcal infections

Bacteraemia The blood was cleared of staphylococci in 5 out of 7 patients with corresponding clinical improvement (Martin, Heilman, Nichols, Wellman and Geraci 1956 b).

Bacterial endocarditis A favourable outcome followed treatment of 2 cases (Pearson *et al* 1956, Cook, Eastman, and Bunn 1957). In others the original staphylococcus disappeared from cultures soon after the beginning of treatment, but one with different cultural characteristics took its place. In still other cases in which the staphylococcus disappeared sufficient time had not elapsed to allow any decision about the eventual outcome.

Osteomyelitis When treatment was not prolonged the results were good,

¹ David and Burgner (1956), Kirby *et al* (1956), Lin and Coriell (1956), Lamson and Romansky (1956), McHardy, McHardy, Ward and Cradie (1956), Martin, Heilman, Nichols, Wellman and Geraci (1956 b), Milberg, Schwartz and Silverstein (1956), Morton, Prigot and Maynard (1956), Mullins and Wilson (1956), Nichols and Finland (1956), Pearson, Somberg, Rosenthal, Lepper, Jackson and Dowling (1956), Pulaski and Isokane (1957 a), Rutenburg, Shapiro and Schweinburg (1956).

cultures from the discharge becoming negative and drainage ceasing. On the other hand, poor results were obtained in 2 patients with chronic infections whose organisms became re-sistant to novobiocin before treatment had ceased (Pulaski and Isokane, 1957 a)

Infections of the hands The majority of these benefited by treatment

Cellulitis This healed well under treatment as a rule

Breast abscess This was usually staphylococcal but was sometimes infected with proteus, or *A. aerogenes* and enterococci. a good response was obtained in approximately half the cases

Furunculosis This healed well under treatment, but when associated with an incurable condition such as leukaemia little response was observed

Carbuncle One case due to *Staph. aureus* healed slowly. In others the effect was notably beneficial

Infected burns When *Staph. aureus* was present in these lesions, it promptly disappeared under treatment while the discharge ceased, making grafting successful

Infections of the skin Some 32 cases of pyoderma were treated, most of them due to *Staph. aureus*, but from a few *Str. pyogenes* or Gram negative organisms were isolated. The response was excellent in 27, good in 3, but those infected with Gram negative bacteria failed to respond satisfactorily

Enterocolitis Thirteen such cases were treated by Martin *et al* (1956 b). The stool cultures lost their staphylococci and diarrhoea subsided. In a patient with carcinoma of the colon, however, it recurred after treatment ceased

Pneumonia In the few cases where staphylococci were found in the sputum, the results varied. They could be good but the antibiotic could not control the infection in the presence of chronic leukaemia

Pneumococcal infections

Pneumonia Although pneumococci were not always the predominant organism in 2 series of 12 and 35 cases treated by Limson and Romansky (1936) and Kirby *et al* (1956), the clinical signs in each patient pointed to their being responsible for the condition treated. All of the patients of Limson and Romansky and of Kirby *et al* (1956) made a good response to novobiocin, definite decrease in fever, cough and toxicity being obvious within 48 hours and all being afebrile within 5 days. The radiographs were clear within 3 to 4 weeks. In those patients who continued to show pyrexia for more than 5 days the presence of sterile pleural effusions or delayed resolution accounted for the delay in recovery, whereas a pure growth of Gram negative rods from the sputum pointed to the supervention of a resistant infection in another old man of 85 who died. Equally good results followed the administration of novobiocin by Pearson *et al* (1956) to 20 patients with pneumococcal, and presumed pneumococcal, pneumonia, for in 18 a good clinical response was recorded

Upper respiratory tract infections Results were found by Pearson *et al* (1956) to be similar to those with pneumococcal pneumonia

Streptococcal infections

A series of 24 children with cultures of *Str. pyogenes* made from their throats were treated by Breese, Disney, and Talpey (1957). Six of these were asymptomatic carriers but the others were suffering from tonsillitis

or pharyngitis, scarlet fever, cervical adenitis, or impetigo and sinusitis. The children were given a syrup preparation, if they were less than 5 years old, containing 125 mg per teaspoonful, or tablets if they were older, the dose varying according to age between 125 and 250 mg in the morning and 250 and 500 mg at night for 10 days. More than half the cultures remained positive during treatment, sometimes disappearing to reappear again later.

The clinical response according to other workers was good whether streptococci were involved in a wound infection in erysipelas or in tonsillitis. However, Pearson *et al* (1956) also pointed out that streptococci had seldom disappeared from the throat by the end of treatment—about the 5th day. Thus recurrences would be possible. A single patient with scarlet fever was treated successfully by Lin and Cornell (1956). In this case haemolytic streptococci disappeared from cultures of throat swabs within 48 hours and did not reappear during therapy.

Enterococcal infections

When associated with pyelonephritis the response was uniformly poor (Pearson *et al*, 1956).

Proteus infections

As some strains of *Proteus vulgaris* were originally found to be relatively sensitive *in vitro* to novobiocin, it was hoped that the antibiotic might have a good clinical effect on infections in which this organism was present. This was so in 1 case of osteomyelitis treated by Pearson *et al* (1956), but in another, though the *Proteus* was cleared from the blood, it persisted in a wound. In a third case it remained unaffected in the blood stream. In fact, in none of these cases did the organism completely disappear. *Proteus* infections of the urinary tract are dealt with on p. 68.

Actinomycosis

Treatment failed in 1 case reported by Pearson *et al* (1956).

Venereal disease

Gonorrhoea

To 27 males with untreated gonorrhoea novobiocin was administered by Willcox (1957 b). On finding 1 or 1.5 G given by mouth 6 hourly in 24 hours insufficient, he then followed it with 4 to 8 G given in 4 daily doses over 2 days. Even with the extended treatment he had 10 failures out of 24 cases followed. Though 2 were probably reinfections the failure rate after a 2 day course of treatment does not compare favourably with results obtained by penicillin, the tetracyclines or chloramphenicol, erythromycin or spiramycin. It also had the disadvantage of producing reactions which, though not serious, were inconvenient, such as slight indigestion or nausea, abdominal discomfort, diarrhoea, or peculiar sensations about the anus.

Syphilis

An attempt was made by Edelson (1957) to see whether novobiocin would have any effect on this disease in its early stages. He had 3 patients all of

whom had lesions which were darkfield positive. Each received 0.5 G dissolved in 500 ml of a 5 per cent dextrose solution intravenously. The infusion was repeated each day till the patients had received 12 to 14 days' treatment. Edelson observed that the sores of 2 patients lost their spirochaetes in 24 to 36 hours and began to heal as rapidly as after penicillin. After a week, however, the healing process slowed down and no visible change was to be seen over the following several days. In the 3rd patient, who had no visible sore but had lymphadenopathy and a rash, there was no change under treatment of the lymphadenopathy but the rash faded slightly during the first 3 days on the palms and soles and disappeared from the body. Thereafter the condition remained stationary until penicillin was substituted for the novobiocin. It would appear then that though novobiocin had an obvious effect over the first few days of treatment, its therapeutic capacity was short lived.

Non gonococcal urethritis

Willcox (1957 c) treated a series of 40 previously untreated patients, care having been taken to exclude those in whom there was a probability of gonorrhoea. Half of them received 250 mg by mouth 4 times a day for 6 days and half received 500 mg similarly administered. When following the cases for up to 3 months Willcox could only record 7 successes in those treated with the lower dose and 9 in those treated with the higher one. Comparing the percentage of failures after treatment with that after other antibiotics, Willcox concluded that novobiocin was the least successful of any.

Conditions where organisms were found without signs of inflammation

Such studies were made by Nichols and Finland (1956) mainly from nose and throat cultures. They were valuable in providing a test *in vivo* in the most suitable subject—man—before therapeutic trials were instituted. The results can be summarized as follows:

| Organism | No of cases where found | No in which organism disappeared | reappeared during or after treatment |
|------------------------------------|-------------------------|----------------------------------|--------------------------------------|
| <i>Staph aureus</i> | 8 | 5 | 1 |
| <i>Proteus</i> | 4 | 0 | 1 |
| <i>E coli</i> | 5 | 1 | 4 |
| <i>Str viridans</i> | 8 | 0 | |
| <i>Str pyogenes</i> | 1 | | 1 |
| Pneumococcus | 1 | 0 | |
| Pneumococcus + <i>Str viridans</i> | 2 | 2 | 1 } |
| | | 1 | |
| Enterococcus + <i>A aerogenes</i> | 1 | | 1 |
| <i>A aerogenes</i> | 8 | * | |

* Appeared in 5 cases, disappeared but returned in 3

Amoebiasis

Twenty one patients who were either cyst passers or suffered from ulceration of the bowel and dysentery were treated with novobiocin. They were given 1.5 to 4 G daily by mouth for courses of 10 days, some receiving a

second course (McHardy *et al*, 1956) As a result of this study it was found that, irrespective of the size of the dose, about half of the patients lost their amoebae and in the other half the protozoa persisted In those with dysentery improvement was experienced in 5 out of 6 cases McHardy *et al* conclude that the effectiveness of novobiocin as an amoebicide was not clinically established

THE TREATMENT OF DISEASES CONSIDERED BY SYSTEMS

Infective endocarditis

No case either acute or subacute has yet been proved to have been cured by novobiocin In some cases the original staphylococcus disappeared from cultures soon after the beginning of treatment, but one with different cultural characteristics took its place In other cases in which the staphylococcus disappeared, sufficient time has not yet elapsed to allow any decision about the eventual outcome In subacute bacterial endocarditis, presumably due to *Str viridans*, early improvement with subsidence of fever occurred after the institution of treatment but the presence of a positive blood culture in 1 case in spite of the favourable signs leads one to suspect that the effect may have been temporary

Infections of the respiratory tract

Acute infections of the upper respiratory tract responded well in the majority of instances even when the infection was mixed

Pneumonia when acute also responded to treatment but when following on such conditions as a cerebrovascular accident, bronchiectasis, or fibrocystic disease of the pancreas, novobiocin made little impression

Bronchiectasis made little or no response to treatment

Two lung abscesses were unaffected by novobiocin

Arthritis

A case of arthritis of the hip with a mixed infection involving *Proteus vulgaris*, *E coli*, an achromobacter, and a staphylococcus showed no effect from treatment

Deep-seated abscesses

Two cases of subphrenic abscess, one due to a *Staph aureus* and the other to *Staph aureus*, proteus, and enterococci, were treated The former made an excellent recovery but in the latter no response to treatment was observed In spite of the usually high resistance of *E coli* to novobiocin a perinephric abscess, from which this was the only organism cultivated, subsided under novobiocin administration

Urinary tract infections

As is the common experience with urinary tract infections which have not responded to treatment in the early stage, the results were very variable Infections with various organisms were treated with novobiocin, but response was uncertain with each type of organism In view of the fact that only 2

per cent of the dose appeared in the urine (Wright, Putnam, and Welch, 1956) expectations of good results in these conditions could not be very high. A summary of the findings made by the workers mentioned above and arranged according to the causal bacteria is given below

| <i>Causal organism</i> | <i>No of cases</i> | <i>Results</i> |
|-----------------------------|--------------------|---|
| <i>Staphylococci</i> | 4 | 1 satisfactory 1—pyuria persisted 2 died—from uraemia or sepsis |
| <i>Proteus</i> | 19 | 9 improved promptly, but 6 only temporarily 7— <i>proteus</i> persisted or treatment failed 3—the result of treatment was indeterminate |
| <i>Aerobacter aerogenes</i> | 9 | 5 improved satisfactorily 2—the result was indeterminate 2—treatment failed |
| <i>E coli</i> | 14 | 6—pyuria cleared but <i>E coli</i> returned in 2 1—pyuria improved 1—the result was indeterminate 6—pyuria persisted |
| No growth on culture | 1 | result satisfactory |

The rather uncertain results seen to be obtained from the treatment of urinary tract infections were subjected to a careful analysis by Trafton and Lind (1957 *a* and *b*). They selected for their study 27 patients who had heavy pyuria and who had shown little or no improvement from therapy with other antibiotics. The conditions they studied were described as cystitis, urethritis, prostatitis, trigonitis, a combination of these inflammatory processes or pyelonephritis, and the infections they chose to treat were those due to *Staph aureus*, enterococcus, *Proteus*, *E coli*, or *A aerogenes*. Treating their patients with moderate doses of 250 mg 4 times a day or 500 mg 3 times a day, they related the sensitivity of the infecting organism and the serum and urine concentrations of each patient with the bacteriological and clinical results. When this was done the correlation was remarkably consistent and though the urine of every case whose infecting organisms were sensitive to concentrations found in his serum and urine was not sterilized, the patient experienced an improvement in his condition with reduction of pyuria. In view of the chronicity of half of the conditions treated these workers showed how consistent results can be, provided one relates the dose to the findings of the laboratory. On p 69 is a list of the infections with the relevant data, each line representing a separate case. It will be seen that no case failed to improve when the concentrations found in the serum and urine were greater than that required to inhibit the infecting organism *in vitro*. When, however, the minimal inhibiting concentration was not attained the result was not so readily predictable.

Infections within the pelvis

Pelvic inflammation Eight cases, 1 due to an enterococcus, some acute and some chronic reacted well to novobiocin.

Endometritis A patient suffering from chronic endometritis was treated by David and Burgner (1956). In spite of the chronicity of the disease the patient made an excellent response to the treatment.

Salpingitis One patient with this complaint, due to a staphylococcus, made a good clinical response.

Meningitis

A case with meningitis secondary to a cranial dysostosis was described. The infection was mixed, *Staph aureus*, an achromobacter, and *Str faecalis* being cultured from the cerebrospinal fluid. The *Staph aureus* disappeared from the fluid but the two other organisms persisted and the patient died. In view of the failure to detect any novobiocin in the cerebrospinal fluid after oral administration to subjects without meningitis, one must infer that the disappearance of the *Staph aureus* in this case must either indicate its extreme sensitivity to the antibiotic or that the permeability of the meninges to novobiocin is increased when they are inflamed. In another patient a good response was obtained but with the help of erythromycin.

| Infecting organism | Sensitivity µg/ml | µg/ml in | | Result | |
|-----------------------------|----------------------|----------|-------|--|----------|
| | | serum | urine | Bacteriological (urine sterilized) | Clinical |
| <i>Str faecalis</i> | 0.78 | 30.0 | 42.0 | + | Cured |
| " | 1.56 | 40.0 | 50.0 | — | " |
| " | 12.5 | 9.5 | 40.0 | + | Improved |
| " | 12.5 | 38.0 | 40.0 | — | " |
| " | 12.5 | 18.0 | 6.0 | — | " |
| " | 12.5 | 14.75 | 11.75 | — | " |
| " | 25 | 6.5 | 6.25 | — | " |
| <i>M aureus</i> * | 0.010 | N R | N R | + | Cured |
| " | 0.078 | 2.0 | 87.5 | — | " |
| " | 0.19 | 50.0 | 40.0 | — | " |
| " | 0.19 | 40.0 | 50.0 | + | Improved |
| " | 0.38 | N R | N R | — | " |
| " | 12.5 | 9.5 | 40.0 | — | " |
| " | 12.5 | N R | N R | — | " |
| " | 50.0 | 15.0 | 10.0 | — | Failed |
| " | 100 | 48.0 | 43.0 | — | " |
| <i>Proteus rettgeri</i> | 6.25 | 15.0 | 10.0 | + | Cured |
| " | 100 | 37.25 | 27.75 | — | Improved |
| <i>Proteus vulgaris</i> | 25.0 | 46.0 | 41.0 | — | " |
| " | 100 | 46.0 | 41.0 | — | Failed |
| <i>Proteus mirabilis</i> | 12.5 | 50.0 | 40.0 | + | Improved |
| <i>Aerobacter aerogenes</i> | 50.0 | N R | N R | — | " |
| " | 100.0 | 20.0 | 87.0 | — | Failed |
| <i>E. coli</i> | 50.0 | 12.5 | 19.75 | — | " |
| " | 100.0 | N R | N R | — | " |

* *Staph aureus* usually in English texts

N R = not recorded

Conclusion

In all these trials, except in those specifically described, the usual dosage given by the different workers was 250 mg 6 to 8 hourly or 500 mg 6 to 12 hourly to adults, while children received approximately 1 G daily or 30 mg per kg of body weight per day.

It is to be noted that in no report concerning these trials was a record of reactions to treatment absent. Most of these were concerned with skin eruptions and eosinophilia, but leucopenia and thrombocytopenic purpura were also occasionally noted. In the latter case, though the patient recovered, the platelets were absent for about 1 month (Breese *et al*, 1957). The trials also support evidence produced *in vitro* of the easy acquisition of resistance in

staphylococci. These organisms were found to become resistant to novobiocin when treatment was prolonged (Pearson *et al.*, 1956, Pulaski and Isokane, 1957 *a*). Pulaski and Isokane (1957 *a*) also expressed this in other words by saying that the antibiotic was ineffective in chronic infections, and Rutenburg *et al.* (1956) summed up their experiences by stating that good results could be expected if a clinical response was observed within the first 48 hours of treatment. Support for this statement was given by the experiences of Cook, Eastman, and Bunn (1957) and High and Huang (1957) in the treatment of acute pneumococcal and staphylococcal infections.

Combinations of other antibiotics with novobiocin

As with oleandomycin it was hoped that combined treatment with another antibiotic with which novobiocin displayed no cross resistance might enable treatment to be continued for longer periods than seemed advisable with novobiocin alone. Lin and Cornell (1957) made studies *in vitro* of the behaviour of novobiocin together with each of the following drugs: sulphisoxazole, penicillin, chloramphenicol, erythromycin, streptomycin, and tetracycline, on 5 different strains of staphylococci. Successive exposures of the staphylococci to the combination in which were increasing concentrations of novobiocin soon revealed that the emergence of resistant strains could be as rapid as that from exposure to novobiocin alone. Unfortunately before the experiments were started the staphylococci were already resistant to penicillin, streptomycin, or to other antibiotics: thus the trial was condemned to failure before it was started.

In an epidemiological study Lepper, Dowling, Jackson, Spies, and Melody (1957) observed the effect on the prevalence of resistant strains of staphylococci when all patients in the Municipal Contagious Disease Hospital of Chicago for whom antibacterial treatment was required received 100 mg of spiramycin per kg together with 30 mg per kg of novobiocin (presumably each day) until total doses of 3 G of spiramycin and 2 G of novobiocin had been taken. To observe the effect on the nose and throat flora in the hospital they made cultures for a period of 6 months from every 4th patient on admission and at weekly intervals thereafter, and on the staff at monthly intervals. By comparing their results with the rate of increase in incidence of strains resistant to spiramycin in a previous study, Lepper *et al.* showed that there was a delay of about 2 months in the appearance of strains resistant to this antibiotic when the combination rather than the single antibiotic was employed. They assumed that a similar delay in emergence of strains resistant to novobiocin would be effected by the combination. This, however, remains to be put to the proof.

Some indication that vancomycin may be of value in combination with novobiocin has been given by Jawetz, Bertie, and Sonne (1957), and Filcott and Hall (1957 *a* and *b*) who concluded from experiments *in vitro*, that synergistic activity between the 2 antibiotics against staphylococci was present in certain concentrations. Neomycin and bacitracin also showed this same propensity. No clinical evidence, however, is yet forthcoming that response to treatment with any of these combinations is more rapid or more certain than to novobiocin alone.

VANCOMYCIN

GENERAL CONSIDERATIONS AND PROPERTIES OF CLINICAL IMPORTANCE

This antibiotic was isolated from *Streptomyces orientalis*, a previously unidentified organism which was obtained from a sample of Indonesian soil (McCormick, Stark, Pittenger, Pittenger, and McGuire, 1956). It was tested first against *Staph aureus* and found to inhibit this *in vitro* with rapidly increasing efficacy as the concentration of the drug was raised. Vancomycin is a white solid complex amphoteric material, very soluble in water and moderately so in aqueous methanol, but insoluble in the higher alcohols. It is precipitated from aqueous solutions by heavy metals, various acids, ammonium sulphate, and sodium chloride. At the time of writing the chemical composition is not thoroughly known. Its antibacterial activity is shown in Table 6. According to Ziegler, Wolfe, and McGuire (1956) the activity of the antibiotic was not significantly altered by changes in pH of the test medium and its behaviour *in vitro* indicated that it was bactericidal for *Str pyogenes* C 203 at as low a concentration as 0.67 μg per ml and for a strain of *Staph aureus* at 1.33 μg per ml. The presence of serum reduced the activity of the antibiotic somewhat and the size of the inoculum had some effect on its action against staphylococci but little against group A streptococci (Kirby and Divelbiss, 1957). In animal protection experiments Geraci *et al* (1957) showed that mice could be completely protected from staphylococcal infections by subcutaneous injections twice a day for 10 days of 1,000 μg . No effect was produced in mice infected with *Toxoplasma gondii*.

Resistance

No cross resistance could be demonstrated in staphylococci which were resistant to tetracycline, streptomycin, penicillin, chloramphenicol, or erythromycin (Kirby and Divelbiss, 1957) nor in 2 strains made resistant to neomycin, bacitracin, and novobiocin (Geraci *et al*, 1957). With *Str pyogenes* acquisition of resistance *in vitro* was by no means marked, possibly because immediate killing of bacteria began as soon as they were exposed to vancomycin. Ziegler *et al* (1956) observed that with *Staph aureus* the degree of resistance developed over 25 transfers was only 4 to 8 fold higher than the original inhibitory concentration whereas a similar trial with penicillin produced a 131,056 fold increase in resistance. Like many antibiotics, it would appear that vancomycin is active only against multiplying bacteria. While Garrod and Waterworth (1956) and Geraci *et al* (1957) confirmed the slow acquisition of resistance *in vitro* of *Staph aureus*, Kirby and Divelbiss (1957) found that in patients under treatment from whom serial cultures were made a few required 3 and 5 μg per ml for inhibition instead of the original inhibitory concentration of 2 μg per ml.

Toxicity

Experimental work carried out by Anderson, Worth, Harris and Chen (1957) showed that in mice and rats the LD₅₀ from intravenous injection was

489 and 319 mg per kg respectively. Tests for chronic toxicity in rats, dogs, and monkeys showed that no visceral or haematopoietic damage had occurred from repeated intravenous injections over periods of up to 29 weeks. There was little or no effect on the blood pressure, respiration, electrocardiogram, intestinal motility of isolated gut, or urinary flow in experimental animals. On attempted sensitization guinea pigs showed no sign of anaphylaxis.

TABLE 6 ANTIBACTERIAL ACTIVITY OF VANCOMYCIN AGAINST ORGANISMS OF IMPORTANCE TO MAN

| Organism | No. of strains | μg per ml required to inhibit growth |
|-----------------------------------|----------------|---|
| <i>Achromobacter</i> | 1 | > 100 |
| <i>Acrobacter aerogenes</i> | 3 | 1.25-> 100 |
| <i>Alkaligenes faecalis</i> | 2 | > 10 |
| <i>Bacillus subtilis</i> | 1 | 0.4 |
| <i>Brucella bronchiseptica</i> | 1 | > 100 |
| <i>Clostridia</i> | 12 | 0.39-5.0 |
| <i>C. diphtheriae</i> | 1 | 0.8* |
| <i>Corynebacteria</i> | 3 | 0.4-0.8† |
| <i>E. coli</i> | 7 | > 10-> 100 |
| <i>Klebsiella pneumoniae</i> | 2 | > 100 |
| <i>Mycobacteria</i> | 10 | 3.1-100 |
| <i>Mycobacterium tuberculosis</i> | | > 500 |
| <i>Neisseria catarrhalis</i> | 4 | 2.5-> 10 |
| <i>Proteus vulgaris</i> | 7 | > 10-> 100 |
| <i>Ps. aeruginosa</i> | 1 | > 100 |
| <i>Salmonella</i> | 2 | > 10-> 100 |
| <i>Sarcina lutea</i> | 1 | 0.39-0.8 |
| <i>Shigella parodysenteriae</i> | | > 10-> 100 |
| <i>Staph. aureus</i> ‡ | 116† | 0.156-6.2 |
| <i>Str. faecalis</i> | 31 | 0.312-12.0 |
| <i>Str. pneumoniae</i> | 7 | 0.29 |
| <i>Str. pyogenes</i> | 33 | 0.156-2.5 |
| <i>Str. viridans</i> | 6 | 0.312-> 10 |

Yeast and filamentous fungi are resistant to more than 100 μg per ml.

No effect observed on vaccinia virus or herpes simplex virus in HeLa cell culture with 50 μg per ml.

* After 48 hours incubation.

† After 72 hours incubation.

‡ Including penicillin, streptomycin, and erythromycin resistant strains.

One hundred and forty-two strains tested by other methods than serial tube dilution, all found to be susceptible to vancomycin by Geraci *et al.* (1957).

From the data of Fairbrother and Williams (1956), Geraci, Heilmann, Nichols, Wellman, and Ross (1957), Griffith and Peck (1956), Kirby and Dangelbiss (1957) and McCormick *et al.* (1956).

Administration

With little absorption into the blood stream following oral administration (Griffith, 1957, Geraci *et al.*, 1957) and the pain following intramuscular injection, the intravenous route was found to be the most suitable way of treating patients with vancomycin. Griffith and Peck (1956) first observed the effect in man of single doses of 50 mg and 100 mg dissolved in 10 ml of 5 per cent glucose solution and injected over 4 minutes. There were no

febrile reactions, changes in blood pressure, pulse, or respiration, nor serious side effects. Occasional tingling of the feet and in 1 case mild abdominal cramping were the only adverse signs. The serum concentrations found 2 and 6 hours after these doses were in the region of 0.5 to 1.6 μg per ml — not sufficiently high in view of the fact that *Staph aureus* usually required between 2 and 3 μg per ml for killing. With a dose of 500 mg and serial assays beginning from 1 minute after the injection, Geraci *et al* (1957) found that levels of 3 μg per ml or over were usually maintained for 6 hours. In 8 healthy young men their findings were as follows:

| <i>μg per ml in blood serum of subjects at time after 500 mg injected intravenously</i> | | | | | |
|---|--------------|---------------|--------------|--------------|---------------|
| | <i>1 min</i> | <i>1 hour</i> | <i>3 hrs</i> | <i>6 hrs</i> | <i>12 hrs</i> |
| Range | 25-40 | 6.4-10 | 3.3-5.2 | 1.0-3.7 | 1.0-1.8 |
| Average | 33.0 | 7.3 | 4.3 | 2.8 | 1.5 |

Repeated intravenous injections were found to maintain the serum levels consistently above the 3 μg per ml limit. In 5 subjects the serum concentrations between doses were

| <i>μg per ml in serum after injections</i> | | | | | |
|--|------------|------------|------------|-------------|-------------|
| <i>No. of injection</i> | <i>1st</i> | <i>5th</i> | <i>9th</i> | <i>13th</i> | <i>17th</i> |
| | 3.6-7.6* | 6.4-9.1 | 6.4-10.5 | 6.4-13.8 | 6.4-11.0 |

* One assay which for an unexplained reason was 17.0 μg /ml, not included.

The method of administration was changed by Kirby and Divilbiss (1957) who found that injection of vancomycin in a concentrated solution over 4 to 5 minutes frequently produced irritation of the surrounding tissues through leakage. The dose was dissolved in distilled water, added to 100 or 200 ml of a 5 per cent dextrose solution and allowed to infuse into the vein over 20 to 30 minutes. After addition to this extent they had very little trouble from irritation and serum levels, after the 4th dose of 500 mg, were maintained at about 10 μg per ml for 12 rather than 8 hours. Concentrations in the serum and urinary excretion of vancomycin after single doses given this way are shown in Fig. 3a and b.

With doses of 1 G and 2 G repeated every 12 and 24 hours respectively, levels of 3 μg per ml or more were maintained throughout the interval between infusions.

| | | <i>μg per ml in patients sera at hours after dose</i> | | | | | |
|---------------|-------------------------------|---|----------|----------|----------|-----------|-----------|
| <i>Dose</i> | | <i>2</i> | <i>4</i> | <i>6</i> | <i>8</i> | <i>12</i> | <i>24</i> |
| 1 G 12 hourly | | | | | | | |
| | 1st | 20 | 10 | | 5 | 3 | |
| | 5th | | | | | 3 and 5 | |
| | 11th | | | | | 5 and 25 | |
| 2 G daily | | | | | | | |
| | 1st | 50 | 33 | | 10 | 10 | 3 |
| | 2nd 6th 8th, 11th and 12th | | | | | | 5-10 |

Distribution in body fluids and tissues

Experimental work in animals by Lee, Anderson, and Chen (1957) showed that half to two thirds of an intravenous dose of vancomycin was recovered within 24 hours from the urine and small quantities were also excreted in the

bile. The presence of the antibiotic in the cerebrospinal fluid was detectable and it was found to pass through the placenta into foetal blood and amniotic fluid. With such indications to guide them, Geraci *et al* (1957) and Kirby

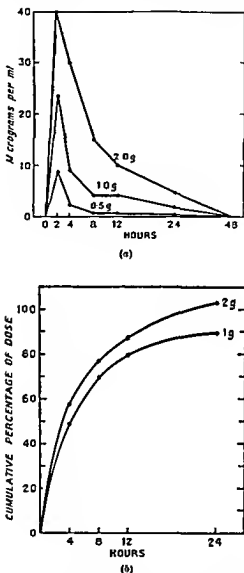


FIG. 3. a. Concentrations of vancomycin in the serum after a single intravenous dose administered over a 20 to 30-minute period.

b. Average excretion of vancomycin by 2 volunteers after administration of 1 and 2 G given intravenously.

(From Kirby and Divelliss *Antibiot. Annu.* 1956: 107.)

and Divelliss (1957) explored the behaviour of the drug in man. Kirby and Divelliss found the urinary excretion to be even higher than that in animals, for 90 to 100 per cent. of single doses of 1 or 2 G were excreted within 24 hours, concentrations in the region of 100 μg per ml. or over being found in the urine during this time. Vancomycin diffused into the pleural fluid in

concentrations approximating those reached in the blood serum and also into pericardial, synovial, ascitic fluids, and bile (Geraci *et al*, 1957). There were, however, no detectable concentrations found in the cerebrospinal fluid in spite of 4.8 to 10 μg per ml being present in the serum at the time of testing. However, in 1 patient of Kirby and Divilbiss (1957) who had a high blood urea nitrogen and whose serum levels reached 100 μg per ml, one tenth of this concentration was found in the cerebrospinal fluid.

Vancomycin combined with another antibiotic

As a basis for clinical trials, Jawetz, Bertie, and Sonne (1957) tested the killing powers of vancomycin together with novobiocin, neomycin, or bacitracin. They found that these combinations sometimes showed greater killing power than either antibiotic alone. Elliott and Hall (1957 *a* and *b*) confirmed the observation of enhancement of activity when novobiocin was added to vancomycin. No clinical trial of vancomycin with any of the above bactericidal antibiotics has yet come to the author's notice.

Complications of therapy

The most frequent and distressing sequelae of treatment were the local reactions in the tissues surrounding the vein through which vancomycin was being administered, or phlebitis in the vein itself. These reactions were reduced in severity when the material infused was diluted (Kirby and Divilbiss, 1957). Geraci *et al* (1957) observed chills approximately 1 hour after injection in a few of their patients but attributed these to the presence of pyrogens as they did not occur following administration of later batches of the material.

Occasional skin reactions, taking the form of morbilliform rashes or diffuse erythema accompanied by pruritus, were seen by Geraci *et al* (1957). Reoal irritation in 2 healthy volunteers as shown by the presence of granular casts, or raising of the blood urea nitrogen from 14 to 20 mg per cent were observed by Kirby and Divilbiss (1957).

CLINICAL TRIALS

Staphylococcal infections

Clinical trials have been mainly concerned with staphylococcal infections, but inasmuch as most patients treated had other associated diseases which were fatal, the results cannot be assessed in terms of full recovery. The 9 patients treated by Geraci *et al*, however, suffered primarily from their staphylococcal infections. These took the form of acute endocarditis, osteomyelitis, cellulitis and ulceration from a spider bite and post operative infections such as that of a wound, an empyema, and 3 cases of ileocolitis. Administration to all patients except those with ileocolitis was by 6 hourly intravenous injections of 500 mg. To the patients with ileocolitis the same dose was given by mouth.

All patients responded well to their treatment, the patient with endocarditis recovered without any sign of recurrence over the following 6

mooths, the 2 patients with osteomyelitis of fibula and tibia respectively were operated on at the same time as vancomycin was begun. Both infections cleared clinically, one, in which the operative wound was closed, healed by primary intention. The post-operative wound was clean and dry by the 4th day of treatment and in the cases of enterocolitis, diarrhoea ceased promptly in 2, slowly in another, but the stool cultures were negative for staphylococci within 24 to 48 hours. It is of some interest to note that though clinical progress was satisfactory in suppurating conditions yet staphylococci continued to be cultured from open lesions for 10 days after the beginning of treatment.

In the staphylococcal infections treated by Kirby and Divelbiss (1957) and by Griffith (1957) vancomycin was notable for its control of the infection while it was continued but for its inability to prevent relapses. This may have been due to the inconvenient method of administration, for the excellent initial response encouraged discontinuance of the drug as soon as the patient seemed well on the way to recovery. In spite of the recurrences however, Kirby and Divelbiss found little evidence of the infecting staphylococci having become more resistant to vancomycin as a result of treatment. The conditions treated included abscesses, superficial and deep seated, earhuncles, furunculosis, osteomyelitis, a post-operative wound infection, empyema, and phlebitis with septicaemia.

Infections other than staphylococcal

The earliest cases treated—those of Griffith and Peek (1956)—were due to *Str. pneumoniae* or haemolytic streptococci. With 50 or 100 mg of vancomycin administered intravenously every 6 to 8 hours, bronchopneumonia was promptly controlled, the temperature falling to normal and the sputum becoming free of pneumococci within 24 hours. The fever from a streptococcal pharyngitis had subsided and the streptococci disappeared from the throat in the same time and a patient with erysipelas lost both fever and leucocytosis promptly, being discharged on his 4th day in hospital.

In a patient suffering from acute recurrent urethritis and complaining of urethral discharge and dysuria the diagnosis of gonorrhoea was confirmed by culture. One hundred mg of vancomycin were injected 8 hourly for 7 days. As a result, the purulent discharge and the dysuria lessened, but the culture remained positive. This infection eventually cleared under penicillin therapy. The organism was not sensitive *in vitro* to vancomycin.

The patients of Kirby and Divelbiss who did not have staphylococcal infections were suffering mainly from pneumococcal or mixed infections. Though they improved under treatment initially, with progressive diminution in purulent sputum and fever and disappearance of Gram positive cocci from smears, either the response could not be attributed to vancomycin because other antibiotics had been used or serious conditions such as severe renal disease, bronchogenic carcinoma or congestive heart failure precluded the patient from making a full recovery.

Of the whole series of 15 patients the results of whose treatment were critically examined by Kirby and Divelbiss 8 died but none of these deaths could be blamed on the failure of vancomycin. In fact it was particularly noteworthy that even in patients with myelogenous leukaemia, diabetes

mellitus, or severe arteriosclerosis, lesions subsided under treatment, unfortunately only to recur when that treatment had ceased

Conclusion

From the limited number of species of bacteria against which it is active, its bactericidal powers and the slow development of resistance to it, vancomycin would appear to be an ideal antistaphylococcal agent. The inconvenience of administration may also be regarded as a factor in its favour, limiting its use to those infections where no other antibiotic has been found effective and thus preventing the encouragement of resistant strains of staphylococci by its widespread use.

Whether the use of another bactericidal agent together with vancomycin would hasten the removal of pathogenic staphylococci from infected tissues still remains to be demonstrated in the clinic.

RISTOCETIN

GENERAL CONSIDERATIONS

Isolated from the fermentation liquor of an unidentified actinomycete called by the authors *Nocardia lurida* and obtained from soil at Colorado Springs, ristocetin gives promise of being of value in the treatment of staphylococcal and other infections due to Gram positive organisms which are resistant to presently available antibiotics (Grundy, Sinclair, Theriault, Goldstein, Rickher, Warren Oliver, and Sylvester, 1957 a). Being an amphoteric substance, containing amino and phenolic groups and sugars, it was isolated as a free base and crystallized as a sulphate (Philp, Schenck, and Hargie, 1957). It is soluble in aqueous solutions but much less so when the pH is neutral. It does not generally go into solution in organic solvents. The acid aqueous solutions are stable but when the pH rises above 7.0 inactivation readily takes place.

Two components, described as A and B, are present in ristocetin. They are distinguished clearly from one another only by paper strip chromatography and paper strip electrophoresis. When crystallized, ristocetin A forms hexagonal prismatic rods and ristocetin B, needle crystals.

Antibacterial activity

Grundy *et al* (1957 a) described the antimicrobial activity as being specific for Gram positive bacteria and mycobacteria but having no action against Gram negative organisms, yeasts, filamentous fungi, or the few protozoa tested. Table 7 gives the range of concentrations found in 1956 to inhibit different organisms. The activity of the ristocetins, according to Grundy *et al* (1957 a), were little affected by the presence of blood or serum or by the pH of the test medium between 5.0 and 7.0. The size of an inoculum did not greatly influence the action of the antibiotics. The action was bactericidal in concentrations close to those which were bacteriostatic, and irrespective

of whether the bacteria exposed were actively multiplying or approaching a quiescent phase

TABLE 7. CONCENTRATIONS REQUIRED TO INHIBIT BACTERIA PATHOGENIC TO MAN

| | No. of strains tested | μg per ml required to inhibit growth |
|---|-----------------------|---|
| Bacteria | | |
| <i>Actinomyces bovis</i> | 1 | 2 |
| <i>Clostridia</i> | 3 | 0.25-2.0 |
| <i>C. pseudodiphtheriticum</i> | 1 | 0.125 |
| <i>C. pyogenes</i> | 1 | 2.0 |
| <i>Diplococcus pneumoniae*</i> | 74 | < 0.1-3.0 |
| <i>Mycobacterium tuberculosis</i> | 13 | 1-5 |
| <i>Nocardia asteroides</i> | 1 | > 50 |
| <i>Staph. aureus</i> (or <i>Micrococcus pyogenes</i> var. <i>aureus</i>) | 63 | 2-50† |
| <i>Str. pyogenes</i> or Group A streptococci | 32 | < 0.1-5.0 |
| <i>Str. viridans</i> | 15 | < 0.1-20.0 |
| <i>Str. faecalis</i> or enterococci | 47 | < 0.1-20.0 |
| Gram negative bacteria | | |
| <i>A. aerogenes</i> | 1 | > 100 |
| <i>E. coli</i> | 2 | > 50 or > 100 |
| <i>Haemophilus influenzae</i> | 4 | > 50 |
| <i>Klebsiella pneumoniae</i> or Friedländer's bacillus | 7 | > 50 or > 100 |
| <i>Neisseria catarrhalis</i> | 1 | > 50 |
| <i>Neisseria meningitidis</i> | 1 | 50 |
| <i>Proteus</i> species | 3 | > 50 or > 100 |
| <i>Pseudomonas aeruginosa</i> | 2 | > 50 or > 100 |
| <i>Salmonella</i> | 3 | > 100 |
| <i>Shigella dysenteriae</i> | 1 | > 100 |
| Fungi | | |
| <i>Candida albicans</i> | 1 | > 25 |
| <i>Trichophyton</i> | 2 | > 25 |
| <i>E. histolytica</i> | 1 | > 50 |
| Trichomonads | 2 | > 50 |

* Or *Str. pneumoniae*

† All *Staph. aureus* tested in purest batches inhibited by 50 μg per ml. or less

From the data of Grundy, Sinclair, Theriault, Goldstein, Rickher, Warren, Oliver and Sylvester (1957) and Romansky, Limson and Hawkins (1957)

Resistance

Among over 400 cultures of staphylococci, streptococci, and pneumococci mainly obtained from hospital sources, no naturally resistant strains were found even when large numbers of bacteria were cultured in the presence of varying amounts of ristocetin. It was possible to induce an 8 fold increase in resistance of a strain of *Staph. aureus* after 25 serial transfers in increasing concentrations of ristocetin but usually the increase was less than this. When it had developed, the cross resistance to ristocetin A and ristocetin B was complete but the strains of staphylococci and enterococci tested developed resistance more readily to A than to B (Grundy, Alford, Rdzok, and Sylvester,

1957) No cross resistance between bacteria resistant to penicillin, streptomycin, the tetracyclines, chloramphenicol, or erythromycin was found by Romansky, Limson, and Hawkins (1957)

Animal protection tests

These were carried out by Grundy *et al* (1957 a) on mice infected intraperitoneally with strains of *Str pyogenes*, *Staph aureus*, or *Dipl pneumoniae*. In each infection the total dose required to protect all infected animals with ristocetin A was approximately 3 times higher than that of ristocetin B. With mouse tuberculosis, however, neither component was effective in controlling this type of infection.

Administration

Experimental work carried out by Grundy *et al* (1957 a) indicated that no absorption took place after oral administration ristocetin would have to be given by the parenteral route. Romansky *et al* (1957) found intramuscular administration too painful to use as a routine method and therefore investigated the absorption which took place from intravenous administration. With repeated doses of 250 mg, 500 mg, or 1 G they assayed the concentrations found in the blood serum. Each dose according to its size was dissolved in 20 to 200 ml of normal saline and injected slowly over periods of 15 to 45 minutes. The concentrations found in the blood serum after the lower doses were sufficient to inhibit *Staph aureus* for 4 or 6 hours and injections of 1 G needed only to be repeated 12 hourly to obtain the same effect. Below are the concentrations they found after different doses.

| Dose | $\mu\text{g per ml}$ in serum at hours after injection | | | | | | |
|--------------------|--|------|------|----|-------|------|----|
| | 1 | 2 | 4 | 6 | 8 | 12 | 14 |
| 250 mg 6 hourly | | | 5 | | | | |
| " 4 " | | | 5 | | | | |
| 500 mg 8 " | | 5-20 | | 5 | 2.5 | 2.5 | . |
| " 6 " | 40 | 40 | 5-20 | 20 | 1.25 | | |
| 1 000 mg 12 hourly | | | | | 10 | 25.5 | 10 |
| " 8 " | | | | | 10-40 | | . |
| " 6 " | . | | 40 | 20 | 10-20 | 10 | . |

1.5 estimates at each time, none made before at least 4 injections had been given

Distribution in body fluids

These had not been extensively investigated at the time Romansky *et al* (1957) reported their experiences in the clinic. However, it was possible to say that the concentration in the pleural fluid of a patient after injections of 500 mg 6 hourly were one quarter of the peak concentrations found in the serum. In the cerebrospinal fluid there was no detectable amount found after the same dose.

CLINICAL TRIALS

All trials that are available at the time of writing are those made by Romansky *et al* (1957) and a further summary of reports made at the Fourth Annual Symposium on Antibiotics, Washington, D C, 1957, by the *Lancet*, 1957. Romansky *et al* confined their studies to the trial of the antibiotic in streptococcal and pneumococcal infections, treating 1 case only which was infected with a staphylococcus. Further trials of ristocetin for staphylococcal infections were carried out in 1957.

Streptococcal infections—bacterial endocarditis

The most impressive early result was that from treatment of an endocarditis due to a microaerophilic enterococcus. The patient, a Chinese of 41, had been ill for 7 weeks without showing a satisfactory response to penicillin, dihydrostreptomycin, and tetracycline. An initial response was made to novobiocin but blood cultures again became positive and remained so for the few days when chloramphenicol was added to the therapy. Ristocetin was then added in the relatively small doses of 250 mg every 6 hours. The last positive blood culture was made on the following day and the temperature subsided within 4 days. In spite of the discontinuance of novobiocin almost immediately and of chloramphenicol in about 1 week, serial blood cultures continued to remain negative. The dose of ristocetin was doubled after a week when chloramphenicol was discontinued, and a week later reduced in frequency to 12 hourly injections. Altogether the patient received treatment for about 3 weeks and in spite of a recurrence of fever due to pulmonary embolism shortly after discontinuance and a further pulmonary episode accompanied by herpes zoster after convalescence, for which he received another week of ristocetin his blood cultures persisted in being negative and he was continuing to do well 6 months after treatment had ceased. Fig 4 shows the patient's temperature, treatment, and effect of ristocetin on blood cultures. A further 5 patients with enterococcal endocarditis have been successfully treated since the report of Romansky *et al* (1957).

A case of bacteraemia due to a microaerophilic enterococcus was also treated successfully by Romansky *et al* (1957) after there had been no response to penicillin given for a period of 5 days. This patient received 1 G every 6 hours by intravenous infusion over 10 days, but in spite of this relatively high dose fever recurred soon after discontinuance of therapy, to be controlled after a second course lasting 5 days.

Pneumococcal infections

Six patients with pneumonia, from whose sputum pneumococci of different types were isolated (pneumococci were also cultured from the blood of 4), lost their fever following intravenous injections of ristocetin. The individual dose varied between 250 mg and 3 G. In those patients to whom as much as 2 to 3 G were administered to start with, the response was excellent, fever subsiding in 4 days at most. In another patient in whom a pleural effusion accompanied the pneumonia the fever did not subside in spite of ristocetin and repeated thoracenteses. Treatment was then changed to penicillin and the patient became afebrile in 5 days.

Staphylococcal infections

Ristocetin is said to have proved useful in staphylococcal pneumonia not responding to other antibiotics. No satisfactory outcome could be ascribed to ristocetin in the treatment of staphylococcal pyoderma. In the single case in which it was used, the antibiotic was given by intra

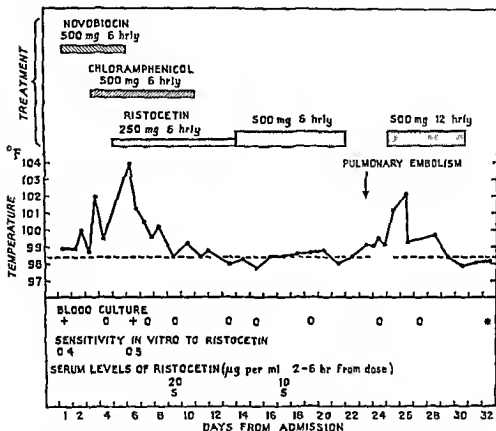


FIG 4 Ristocetin and endocarditis—course and treatment after admission in a 41 year old patient with bacterial endocarditis due to a microaerophilic streptococcus. This patient had already received large doses of penicillin and streptomycin elsewhere without ridding his blood stream of the streptococcus. The 2 peaks in the temperature curve correspond with an attack of mumps and a pulmonary embolus respectively.

* Subsequent blood cultures for 8 weeks were sterile.

(From Romansky, Linson and Hawkins *Antibiot Annu* 1956-7 706)

muscular injection, a procedure so painful that tetracycline was substituted for it.

Non-specific respiratory tract infections

In 5 such cases, 4 of pneumonia and 1 of bronchitis, an excellent response followed intravenous injection but intramuscular administration gave rise to much pain at the sites of injection, the patients only becoming afebrile after they received their therapy intravenously. In the 5th patient, however, who began with intravenous therapy, phlebitis developed at the site of injection and, whether because of this or a concomitant pleural effusion, the fever did

not subside until after erythromycin had been substituted for ristocetin, (Romansky *et al*, 1957)

No reports of the effect of ristocetin in tuberculosis have come to hand as yet

Complications of therapy

The difficulties encountered by Romansky *et al* in the treatment of their 16 patients amounted to 1 instance of phlebitis and the experience by the patients of considerable pain on intramuscular injection. The latter can be avoided but as the intravenous route is the only one left by which administration can be carried out, dilution of the antibiotic and adjustment of the infusion to a pH of 7.0 is indicated to forestall, if possible, irritation of the vein walls. Transient leucopenia was also occasionally observed.

STAPHYLOMYCIN

De Somer and Van Dyck (1955) described staphylomycin as an antibiotic isolated from an unidentified *Streptomyces* obtained in Belgian soil and particularly active against haemolytic streptococci and staphylococci. Its toxicity in animal experiments was low and excellent protection was given to mice infected with *Str. pyogenes*. No cross resistance was found with the antibiotics penicillin, streptomycin, the tetracyclines, chloramphenicol, or erythromycin. With streptogramin (Charney *et al*, 1953), however, cross resistance was complete. Resistance was induced in a strain of staphylococcus after 31 passages but the resistant mutant was less virulent than the original sensitive strain and it was no longer haemolytic.

By paper chromatography it was possible to separate the antibiotic into 3 components, described for convenience as M I, M II, and S (Vanderhaeghe, Van Dyck, Parmentier, and de Somer, 1957). These workers claimed that the melting points, optical rotation, and ultra-violet spectra were sufficient to distinguish these products from those found in other antibiotics. The maximum antibacterial activity against *Staph. aureus* was obtained with a mixture of 70 per cent M I and 30 per cent S. No action against Gram-negative bacteria or fungi was seen in solutions containing as much as 100 µg per ml but *Myc. tuberculosis* H37 Rv was inhibited in low concentrations by a combination of 90 per cent M and 10 per cent S (Van Dyck, Vanderhaeghe, and de Somer, 1957).

Clinical trials were undertaken by de Somer and Van de Voorde (1957). Fifty patients suffering from various infections with *Staph. aureus*, mostly resistant to available antibiotics, were treated. The conditions included post-operative infections, cystitis, chronic bronchitis, abscess, tracheobronchitis in a case of poliomyelitis, aspiration pneumonia, enteritis, paronychia with lymphangitis, suppurating fistulae after subperiosteal resection of the tibia, subdiaphragmatic abscess with enteritis, and empyema with cerebral metastases. Angina in which a β haemolytic streptococcus was also found completed the list.

The treatment consisted of administering 1.5 to 2 G daily by mouth in

4 doses and/or local applications of a suspension at pH 7 containing 10 mg per ml (This remained stable on storing for 1 week.) The results in each case were described individually. Twenty five patients received staphylomycin by mouth. Following its administration the patients' temperatures fell to normal within 24 hours to 5 days, depending on their previous condition. The intestines—there were 9 cases of enteritis—were freed of staphylococci, abscesses were sterilized and persistently open and infected wounds healed. Three cases did not recover, 2 who had septicaemia showed no improvement, 1 dying from an embolus. Another patient with chronic bronchitis died of anoxia. After death many minute abscesses were found in the bronchi.

Of the 10 patients who were treated both by mouth and locally, 2 died—1 from septicaemia due to a pseudomonas and another from heart failure. In the others suppuration ceased, the lesions were sterilized or the staphylococci reduced in number. In those who received local treatment only, 15 in number, resorption of pus with recalcification of bones followed the sterilization of lesions. There was complete healing in 14 patients, the 1 exception being in a wound in which *Ps. pyocyanea* appeared.

The reactions from treatment were limited to a rash and dizziness in 1 patient and some nausea and vomiting in another. The blood cell counts remained unchanged during treatment, or improved.

Thus, among 50 patients, failures to improve under treatment amounted to 6, some of these being due to the supervention of a resistant infection and others, such as heart failure or embolus, were not directly due to the persistence of staphylococcal infection. In the great majority staphylomycin had a demonstrable effect on the staphylococcus, the healing of wounds and the subsidence of fever and symptoms.

AMPHOMYCIN

A crystalline polypeptide antibiotic, active against Gram positive bacteria, amphomycin was isolated from culture broths of a *Streptomyces* called *canus* by Heinemann, Kaplan, Muir, and Hooper (1953). It was crystallized and found to be an acidic polypeptide soluble in water and the lower alcohols, highly surface active and stable for a month in aqueous solutions with a neutral pH at room temperature. The pathogenic organisms against which it was particularly active *in vitro* that is, those inhibited by concentrations of 2.5 µg per ml, were as follows¹

| | |
|---|--------------------------------|
| <i>Mic. pyogenes</i> var <i>aureus</i> | <i>Bacillus anthracis</i> |
| <i>Str. pyogenes</i> and other streptococci | <i>Bacillus subtilis</i> |
| <i>Dipl. pneumoniae</i> | <i>Corynebacterium xerosis</i> |

It had little or no activity against *Salm. typhosa*, *E. coli*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, or *Candida albicans*.

¹ Confirmed by Reedy and Shaffer (1957) who found that some strains of streptococci required 12.5 and some *Staph. aureus* 62.5 µg per ml for inhibition.

One advantage this antibiotic possessed was its ability to inhibit 10 low concentrations strains of *Staph aureus* which were resistant to penicillin, chlortetracycline, and oxytetracycline. The disadvantage of applying this drug to man, however, was revealed by Tisch, Hoekstra, Fisher, and Dickson (1955) who found that little was absorbed in experimental animals after oral administration. Intravenous or intramuscular injections of more than 50 mg per kg, although producing high blood concentrations, were apt also to produce renal damage and cardiac effects. It was still possible to try topical application and, in view of its being highly surface active, this method of administration held out hopes of being satisfactory. In 1956 Cronk and Naumann applied ointments containing 5 mg per G of amphomycin combined with or without a similar concentration of neomycin to 28 patients suffering from skin diseases. These consisted mainly of acne vulgaris, impetigo, infected cysts, or contact dermatitis. The organisms found in the lesions were *Staph aureus*, *Proteus vulgaris*, *Bacillus subtilis*, *H influenzae*, haemolytic and other streptococci. Obviously a number of these species were unsusceptible to amphomycin, as sensitivity tests *in vitro* showed, and for this reason it was hoped that neomycin would be of benefit. However, in spite of applications twice a day carried on for 2 to 8½ weeks, there was no appreciable effect on the acne, but the majority of patients with impetigo improved promptly as also did those with contact dermatitis.

There was no evidence of skin sensitization or of irritation from the ointments.

CHAPTER 4

ANTIBIOTICS INHIBITORY TO VARIOUS BACTERIA, INCLUDING GRAM-NEGATIVE ORGANISMS

FRAMYCETIN, SYNAMMATIN B, ALBOMYCIN, CYCLOSERINE

FRAMYCETIN

OBTAINED from a *Streptomyces lavendulae*, framycetin or soframycin was prepared in France by Decaris in 1947 (Decaris, 1953). It is a tasteless substance, readily soluble in water, and the solutions are stable. It is relatively insoluble in organic solvents and its empirical chemical composition consists of C, 46.6, H, 7.5, W, 12.8, O, 33.1 per cent (Shidlovsky, Marmell, and Prigot (1956)). Its antibacterial range covers both Gram positive and Gram negative bacteria, including *Escherichia typhosa*, *Salmonella paratyphi* A and B, shigellae, *E. coli*, proteus and pseudomonas species. Staphylococci, including those resistant to penicillin, are sensitive to it and some strains of *Mycobacterium tuberculosis* are also inhibited by its action. When given parenterally framycetin is toxic to the kidneys and to the 8th cranial nerve (Massenat-Deroche, 1954) but its poor absorption from the gut enables it to be given without harm for infections arising within the lumen of the gastro intestinal canal.

CLINICAL TRIALS

A number of workers have employed framycetin, alone or with other antibiotics, for the treatment of gastro enteritis in infants, and have found it useful.¹ Laplane *et al* (1955) treated 26 infants with gastro intestinal symptoms, 7 of whom were severely ill, and from all of whose faeces specific strains of coli bacilli were isolated. Stool cultures in the majority became negative for the particular strain of pathogen being excreted within 48 hours of one or two 3 day courses of treatment. In a further 8, 7 to 8 days' treatment sufficed to free the stool cultures, 1 case receiving also the help of chloramphenicol.

These earlier results were confirmed by Schneegans *et al* (1956). One hundred to 250 mg administered in 2 doses daily sufficed to clear the stools of 32 out of 33 cases infected with *E. coli* 111B4 or *E. coli* 55B5 in approximately 5 days, the stools having become normal in consistency within 24 hours of beginning treatment, in some instances without the necessity of modification of the diet. Schneegans *et al* compared these clinical and bacteriological results with those obtained in other cases with chlortetracycline, chloramphenicol, and neomycin. No series escaped relapses or failures but the duration of the illness under treatment with framycetin

¹ Cathala, Poupinet, Janet and Naudot (1954). Laplane, Debray, and Duché (1955), Marquézy, Debray, Leveau, Grenier and Lepage (1955). Pierret, Breton, Gaudier, and Prouvost (1956). Schneegans, Haarscher, and Levy (1956).

compared favourably even with neomycin. They considered its poor absorption into the blood stream compared with that of chlortetracycline and chloramphenicol was another characteristic in its favour. Still another group of workers—Pierret *et al*, 1956—chose to add neomycin to their therapy for infantile gastro enteritis, the doses being framycetin 100 mg and neomycin 0.25 G administered for 5 to 8 days. Good results were obtained in most cases but even with the addition of this bactericidal drug the results were not uniformly so. In drawing conclusions as to the value of framycetin, therefore, one must take into account that where so many different organisms are liable to be partly responsible for the condition of the patient, its failure to bring about recovery in all those under treatment is not necessarily an indication of inefficiency.

In another series of 60 patients receiving chloramphenicol in addition to framycetin, the latter antibiotic was prescribed in doses of 0.125, 0.25 or 0.5 G per 24 hours in 1 or 2 doses for approximately 6 to 10 days (Marqu  zy *et al*, 1955). The coliform pathogens disappeared from the stools of 52 of the patients, including carriers, major and minor forms of gastro enteritis, and neurotoxic cases and the clinical results were immediately favourable. In the remainder treatment had to be prolonged, but the result was unfavourable in 1 patient only. Sensitivity tests of 51 of the strains isolated showed that they were susceptible to framycetin irrespective of their reaction to chloramphenicol. In adult patients with normal gastro intestinal canals, Shidlovsky *et al* (1956) investigated the action of the antibiotic on the bowel flora. Groups of 5 individuals each received 0.25 G 3 times in 1 day or 0.5 G 4 times during the same period. When colony counts were made from 1 G of wet stool before and after treatment there was no doubt about the reduction in the number of bacteria in the treated patients. Below are the figures before and after the doses described.

| Dose | no of pat ents | Bacterial colony count (mill ions) | | |
|-------------------------|-------------------|------------------------------------|--------------------|----------------------------|
| | | before treatment | after treatment | percentage of reduction |
| 0.25 G 3 times in 1 day | 5 | 10-660 | < 1-4 | > 99 |
| 0.5 G 4 | 5 | " 600 | < 1-5 | > 99 |

There was a decrease in both Gram positive and Gram negative bacteria. *P. coli*, *pseudomonas* and *proteus* species although present before treatment were not isolated in the first specimens examined 18 to 24 hours after it had been begun. After cessation of treatment, however, the bacteria originally present gradually returned and within 48 hours had reached higher numbers than were present before framycetin had been taken. A study was also made of the presence of yeasts and fungi. These if anything increased in number during treatment while anaerobic spore formers were little affected. No ill effects followed the administration of the antibiotic for this limited period of time.

Shidlovsky and Prigot (1957) made a second trial of the effect of combining framycetin with nystatin in an effort to reduce not only bacterial flora but also yeasts in the faeces. By administering framycetin 500 mg together with nystatin either 500 000 or 1 000 000 Units 4 times a day for 24 hours and again examining wet stool samples before and after beginning treatment, they found a reduction in flora of the order of 90 to 99 per cent 24 to 48 hours

from beginning administration. This time the organisms affected were enterococci and yeasts as well as *E. coli*, proteus species, *Pseudomonas*, *Paracolobactrum*, and *A. faecalis*. Shullovsky and Prigot concluded from their findings and from the absence of side effects that framycetin with nystatin would be a suitable method of preparing the bowel for surgery.

A trial was made of the effect of framycetin in staphylococcal infections by Weber (1953). In 48 cases of long standing tuberculous fistulae secondarily infected with staphylococci and refractory to previous treatments with other antibiotics, Weber injected the drug directly into the fistulae daily, 25 to 50 mg in 5 to 15 ml of physiological saline being given. When joints were involved they were appropriately immobilized and the treatment which the patients were already receiving, such as streptomycin and isomazid, was continued. Results were equivocal for the fistulae closed in 15 cases and remained healed for at least the following 3 months, in 9 others healing followed some further surgical intervention, in 12 the fistulae were greatly improved although not fully healed while in 9 they broke down again within 3 months of healing.

SYNNEMATIN B

An Antibiotic Active against Salmonella of Typhoid Fever

This antibiotic was first described by Gottshall, Roberts, Portwood, and Jennings (1951). It was prepared from a mould belonging to the genus *Tilachelidium* and was found by these workers to be effective *in vitro* against a number of species of *Salmonella* including *Salm. typhi* and also against brucella. Other organisms which were very susceptible were *C. diphtheriae* (0.25 Units per ml), *Str. pneumoniae*, *Staph. aureus*, and *Proteus* species. An occasional strain of *Shigella* and some haemolytic streptococci were also sensitive. Although not obtained in the pure form, a preparation containing 8 to 32 Units per ml¹ (1 Unit = the amount required to inhibit *Salm. typhi murium* for 24 hours) was able to free chick embryos of infection with *Salm. pullorum* after inoculation of a culture containing 1,000,000 organisms, whereas no controls were free after inoculation of a culture 100 times as dilute. Mice were similarly protected from infection with *Str. pneumoniae*.

Its activity was reduced by the presence of serum, but the size of the inoculum had little effect.

Two components of the synnematin were identified by Olson, Jennings, and Junek (1953), of which the second—B—was thought to have the greater significance in clinical application, for it was this one which showed its activity particularly against *Salm. typhimurium*. Confirmation of its activity was made *in vivo* when Olson and Jennings (1954) treated mice infected with this organism by subcutaneous injection. The animals were freed from infection as judged by cultures made from heart blood and organs. Further work on the sensitivity *in vitro* of strains of *Salm. typhi* was carried out by Olarte and Figueredo (1955). Forty were tested and of these 13 were inhibited.

¹ Synnematin can now be purified to yield over 600 Units per mg (Clark, Fricke, and Lanus, 1957).

by 0.5 Units per ml, 25 by 1 Unit per ml, and 2 by 2 Units per ml. The next step in testing the antibiotic was its trial in man. In this Benavides, Olson, Varela, and Holt (1955) made trial of 16 cases, 15 infected with *Salm typhi* and 1 with *Salm paratyphi*. All strains were inhibited by 0.5 to 2 Units per ml. The patients were given 4 hourly intramuscular injections amounting to between 80 mg and 350 mg per kg of body weight per day. A loading dose was early employed but this was followed by chills and hypothermia and so was omitted later. Treatment was continued for 12 to 14 days. Responses were good in 12 patients, with fall of temperature within 4 to 10 days of beginning treatment, marked clinical improvement and disappearance of the salmonella from the blood and faeces—a condition which was maintained in convalescence. In the remainder of the patients the fall of temperature was irregular or slow, 3 relapses occurring after initial improvement. Observations were made on the rapid excretion of the drug in the urine, and in the blood levels after given doses. These were as follows:

| Dose | Units per ml in blood between 1 and 4 hours after dose |
|----------------------|---|
| 80 mg per kg per day | 0.5–2.2 |
| 160 " | 0.5–5.8 |
| 320 " | 1.2–8.0 |

Like penicillin oral administration of synnematin B was followed by poor absorption but intramuscular administration of aqueous solutions and suspensions in oil were well absorbed, producing readily recognizable levels in blood and urine (Sylvester, Kirchmeyer and Farago, 1957).

Comparison of the effect of synnematin B with that of chloramphenicol in experimental animals has been attempted by Hobby, Pikula, Vrabec, Daly, Sarrocco, and Lenert (1957). They did not lead to clear cut conclusions but experiments did show however that synnematin B was capable of reducing markedly the number of *Salm typhimurium* in the organs of infected mice.

The question arose whether synnematin B was not identical with one of several antibiotics obtained from a different source—sewage material issuing from Cagliari, Sardinia and collected by Brotzu in 1948 (Florey, 1955). This was termed cephalosporin N and was considered to be chemically identical with synnematin B both antibiotics being a derivative of penicillin provisionally described as aminocarboxy butyl penicillin (Abraham, Newton, Schenck, Hargie, Olson, Schourmans, Fisher, and Fusari, 1955; Abraham, Newton, Crawford, Burton, and Hale, 1953).

ALBOMYCIN

Albomycin was brought to the notice of the medical world in 1951 (Dol-rochotova, Judinzev, Krechmer, Valtor, and Buandina, Shapiro, and Shorin). For a description of its properties the author has depended on translations: the descriptions given in English by Gause (1955), and the leading article in the *British Medical Journal* (1955, ii, 1198). The antibiotic was derived from cultures of a new species of streptomycetes called *Actinomyces*

subtropicus It was found to inhibit Gram positive cocci readily, particularly the pneumococcus and staphylococcus, the latter was inhibited in the extreme dilution of 1 in 700 million,¹ a concentration that is 10 times more dilute than that at which penicillin is active. Haemolytic streptococci, however, are rarely sensitive (Garrod and Waterworth, 1956). Gause claimed that it was also effective against certain Gram negative bacteria such as the coli dysentery group and Friedlander's bacillus. Its chemical nature was described by Brazhnikova, Lomakina, and Muravieva (1954), but its isolation has presented a number of difficulties. It is a peptide containing 1 unidentified and 6 identified amino acids and 4.16 per cent of iron. When this latter constituent is removed from the molecule the antibacterial activity is reduced 14 or 15 times but this can be restored again by simply adding a drop of 5 per cent solution of ferric chloride. Its activity is also lost under anaerobic conditions, and reduced in 10 per cent blood serum. However, 1 per cent casein or a 10 per cent solution of egg albumen do not affect its activity. It forms salts with various acids. The sulphate of albomycin is an amorphous red powder which is easily soluble in water, only slightly so in methanol and insoluble in other organic solvents. Later investigations were carried out by Stapley and Ormond (1957) into the comparison of albomycin with grisein, an antibiotic reported on by Reynolds, Schatz and Waksman (1947) whose clinical trials were abandoned owing to the ease with which resistance was induced in bacteria exposed to its action. Stapley and Ormond considered that these 2 antibiotics were related owing to strains of *E. coli* being mutually cross resistant. Further comparison by paper chromatography and column partition chromatography indicated that the 2 were chemically similar and identical with respect to their antimicrobial activity. Such a conclusion from the available data was confirmed by Waksman (1957). From the ascending and descending paper chromatography patterns 4 antibiotically active substances were distinguished in both albomycin and grisein, each of differing activity and stability from the others. It is possibly the varying proportions of these components which may explain the differences found in the antibacterial activity of albomycin by Gause (1955) and Garrod (1956).

Antibacterial activity

The following bacteria were found sensitive to albomycin

| | |
|---|-----------------------------------|
| <i>A. aerogenes</i> | Pneumococci |
| <i>B. subtilis</i> | <i>Sarcina subflava</i> |
| <i>E. coli</i> | <i>Shigella dysenteriae</i> |
| <i>Haemophilus pertussis</i> ² | Spirochaetes |
| Klebsiella | <i>Staph. aureus</i> ³ |
| Meningococci ² | <i>Str. pyogenes</i> ³ |

Organisms which are insensitive are

Listeria
Mycobacterium tuberculosis
Bacillus mycoides

¹ According to Garrod (1956) the activity varies considerably with the size of the inoculum

² Less sensitive than the other listed species

³ Some strains only sensitivity not confirmed in all strains tested by Garrod (1956)

Resistance

Albomycin resistant strains of staphylococci had already been met with in clinical practice by 1955, but at that time they constituted only 1 per cent of strains tested, but by 1956 Garrod and Waterworth had remarked on the 'unexamined facility' with which a small minority of any population of staphylococci could become resistant to the antibiotic. There was no cross resistance between penicillin resistant and albomycin resistant strains. The mechanism of the antibacterial action of this drug has been shown to be typically bacteriostatic, and to be dependent on the presence of oxygen, irrespective of the concentration of the drug (Shorin and Sazukin, 1954).

Experimental trials in mice (Shorin, 1951) showed that 100 per cent survival from 500 000 lethal doses of pneumococci could be obtained with a single subcutaneous injection of 750 000 Units per kg of weight. By contrast a single dose of penicillin could only secure a 20 per cent survival amongst similar animals. Good therapeutic results were also claimed for the treatment of experimentally induced staphylococcal and haemolytic streptococcal infections, and also infections with dysentery bacilli. Infections of the blood and brain induced in guinea pigs with *Spirochaeta sodanum* were eradicated by injections of 150 000 Units per kg of body weight daily for 14 days. This again compared favourably with the results produced by penicillin in similar infections. Only 25 000 Units¹ per kg of body weight given subcutaneously were required to secure 100 per cent survival in mice infected with 50 lethal doses of Friedlander's bacillus, 500 lethal doses required 4 times as much albomycin.

Toxicity

The absence of toxicity had been demonstrated in animals and man. So far, it has not been possible to find a lethal dose for mice, rabbits, cats, or guinea pigs. When injected subcutaneously or intravenously, mice have tolerated 50 million Units¹ per kg of body weight. Large intravenous doses do not affect the heart, blood pressure, or respiration, nor is there any cumulative toxicity on repeated administration. Intrathecal injections were not accompanied by any side reaction, nor did histological examination of the organs, including haemopoietic tissues of laboratory animals treated with large doses daily for 30 days, reveal any abnormalities. In man, Gause claimed, extensive clinical trials had demonstrated the lack of any harmful effects. When injected into the body, albomycin is partially but reversibly bound to serum proteins. The complex is capable of dissociation *in vivo* but not *in vitro*. It is therefore impossible by the usual methods of titration to detect its concentration, unless this is 150 Units per ml or more. However, if serum containing this bound albomycin is injected intraperitoneally into mice infected with pneumococci or dysentery bacilli it exerts some protective effect. Excretion via the kidneys is not great (Judinzev, 1951). If, however, injections are repeated the amount excreted by the kidney increases sharply, since once the serum proteins are saturated, albomycin remains free in the serum.

¹ 1 Unit = the minimum amount per ml which will inhibit a staphylococcus by weight this is probably about 0.0014 µg. Fifty million Units per kg would therefore only be 1.4 mg in a 20 g mouse.

Clinical Trials

Albomycin had been used with success in the treatment of pneumonia in young children, in the septic complications of dysentery and measles, and in various septic conditions caused by penicillin resistant bacteria (Gamburg, 1951, Sokolova and Smirnova, 1953 Dobrochotova, 1951, Krechmer, *et al* 1951, Raikher, and El man, 1952) Good results in children with pneumococcal meningitis said to be resistant to penicillin have been obtained by intrathecal injections of 100,000 to 200,000 Units There were no side reactions Gilevich (1953) found that relapsing fever responded favourably to albomycin For adults the dose used was 3 million Units intramuscularly twice a day for 7 to 12 days Peritonitis and other surgical infections were treated by Zemskov (1953) whose cases showed that the antibiotic exerted some favourable action, and Berent and Gilman (1954) used albomycin to treat infections due to penicillin resistant bacteria

One of the difficulties in assessing this drug is the absence of any supplies with which to confirm its reported therapeutic effects One must wait until such supplies are available before pronouncing categorically on the value of albomycin

CYCLOSERINE

An Antibiotic with a wide antibacterial range

Cycloserine, or PA94 (known by the trade names of *Seramycin* and *Oxamycin*), was derived originally from *Streptomyces orchidaceus* by Harned, Hidy and La Baw (1955) It was prepared from *Streptomyces garyphalus* obtained from soil both in Guatemala and in the State of New York (Harris, Ruger, Reagan, Wolf, Peck, Wallick, and Woodruff, 1955) It also seems to have been produced from other streptomyces—*Streptomyces lavendulae*—by Shull and Sardinas (1955) It has the chemical structure of D 4 amino 3 isooxazolidone, is amphoteric, very soluble in water, moderately so in most organic solvents, and is highly diffusible It has no definite melting point but decomposes at 153° C In aqueous solution or at an acid pH it is rather unstable but little affected by media between pH 4.5 and 8 The antibacterial activity of this antibiotic was demonstrated by Harris *et al* (1955) with the following organisms *E. coli*, *Klebs pneumoniae*, *Ps aeruginosa*, *Salm schottmulleri*, *Salm typhosa*, *Shigella dysenteriae* (sbiga), *Staph aureus*, *Str pneumoniae*, and a streptococcus resistant to streptomycin Besides these organisms, Cuckler, Frost, McClelland, and Solotorovsky (1955) found it effective against rickettsiae and protozoa, and Cummings Patnode, and Hudgins (1955) showed that *Mycobacterium tuberculosis* was susceptible to its action irrespective of whether it was sensitive or resistant to streptomycin or isoniazid Table 8 shows the minimal inhibitory concentrations in serial dilution tests

TABLE 8 ANTIBACTERIAL ACTIVITY OF CYCLOSERINE AGAINST ORGANISMS PATHOGENIC TO MAN

| Organism | No. of strains | μg per ml required to inhibit growth |
|--------------------------------|----------------|---|
| <i>Aerobacter</i> | 10 | 100-500 |
| <i>C. diphtheriae</i> | 5 | 6.25-50 |
| <i>E. coli</i> | 10 | 100-125 |
| <i>E. runopathiae</i> | 1 | 50 |
| <i>Klebsiella</i> | 10 | 25-500 |
| <i>Mycobacter</i> | 5 | < 62.5-125 |
| <i>Mycobacter tuberculosis</i> | 1 | 10-20* |
| <i>Pasteurella</i> | 1 | 50 |
| <i>Proteus</i> | 10 | 125-500 |
| <i>Pseudomonas</i> | 10 | 100-1 000 |
| <i>Salmonella</i> | 10 | 50-100 |
| <i>Shigella</i> | 11 | 50-100 |
| <i>Staph. aureus</i> | 14 | 6.25-100 |
| <i>Streptococci</i> | | |
| <i>Str. faecalis</i> | 10 | 50-125 |
| enterococci | 10 | 50-250 |
| <i>Str. pneumoniae</i> | 7 | 6.25-125 |
| <i>Str. viridans</i> | 3 | 50-125 |

From the data of Cummings, Patnode and Hudgins (1955), Cuckler, Frost, McClelland, and Solotorovsky (1955).

* According to media used.

Activity *in vivo*

Mice were protected from experimental bacterial infections by small oral doses of 1 to 2.5 mg. per mouse. The action of the drug *in vivo* was demonstrated against *Borrelia burgdorferi*, the spirochaete of relapsing fever; cycloserine was one tenth as active as penicillin. Against *E. histolytica* in young rats the activity of the drug was low and no demonstrable effect was produced on infections with coccidioides. *Trypanosoma brucei*, *Trichomonas foetus*, and *Plasmodium gallinaceum*. Cycloserine had no antihelminthic activity against infection with *Schistosoma mansoni*, *Syphacia obvelata*, or *Aspicularis tetraptera* in mice (Cuckler *et al.*, 1955).

One great advantage that this drug possessed was its property of acting synergistically with other antibiotics. Two groups of early workers testified to this. Harris *et al.* (1955) observed this phenomenon *in vitro* with penicillin, bacitracin, the tetracyclines, and chloramphenicol, and Cuckler *et al.* (1955) noted it *in vivo*.

Toxicity

Both acute and chronic toxicity of cycloserine as judged by its behaviour in animals are low (Welch, Putnam and Randall, 1955), but in man evidence of a deleterious effect on the central nervous system has been observed, as manifested by headache, vertigo or drowsiness, and occasionally convulsions (Epstein, Nair, and Boyd, 1955). It was found that during administration of the drug the protein in the cerebrospinal fluid tended to rise abnormally and reached 80 to 93 mg. per 100 ml. (McDermott, 1955, personal communication). In a United States Public Health Service investigation (1956)

a proportion of patients being treated for tuberculosis who received graded doses of 0.25 G or 0.5 G twice a day, 0.5 G once a day or 1 G once every 2 days manifested convulsions, mental disorders, motor disorders, somnolence and dizziness, or fever and chills during each scheme of treatment except that in which 0.25 G was given twice a day. This led the Council on Drugs (1957) to recommend that initially the dose should not exceed this amount in the treatment of any infection.

Administration

In spite of the ability of the drug to inhibit different types of organisms and its protective effect against infections induced by these, the serum concentrations detected after administration of the usual doses of 0.25 to 1 G were often lower than the concentrations required to inhibit the bacteria in question *in vitro*. Cycloserine is readily absorbed when given by mouth, serum concentrations of 30 µg per ml or over being attained from 4 to 24 hours after a dose of 0.25 G, while 1 G produced a serum concentration of over 100 µg per ml. When administered 6 hourly the following average values were obtained just before the next dose:

| Dose, 6 hourly | 6 | 12 | µg per ml at hours from first dose | | | | | | | |
|-------------------|------|----|------------------------------------|----|----|----|----|----|----|----|
| | | | 18 | 24 | 30 | 36 | 42 | 48 | 54 | 60 |
| 0.5 G | 10.1 | 18 | | | | | 32 | 32 | 34 | 43 |
| 0.75 G | 13.7 | 24 | 30 | 32 | 38 | | 48 | 42 | 46 | 54 |

Rise in blood concentrations was slight or nil after the 60th hour.
After Welch, Putnam and Randall (1955)

When Robinson, Morgan, Richards, Frost, and Alpert (1955) estimated the blood levels in some patients they found roughly equivalent concentrations to those above after similar doses. They gave the following figures:

| Dose | No. of cases | Range of µg per ml in serum |
|-------------------|--------------|--------------------------------|
| 0.2 G twice a day | 4 | 3-32 |
| 0.2 G 6 hourly | 2 | 7.4-40 |
| 0.4 G , | 1 | 8-19 |
| 0.6 G , | 2 | 4-42 |

Excretion

Excretion in the urine. Conzelmann (1955) administered 50 to 100 mg per kg of body weight intramuscularly and by mouth to rhesus monkeys and measured the urinary output. In the first 24 hours 80 per cent of the dose was excreted, but measurable amounts were still being passed 48 and 72 hours after administration. In man the urinary excretion, according to Welch *et al.* (1955), is approximately 60 per cent of the dose taken, confirmed by Conzelmann (1955), and continues for 3 days after the last dose. Average values are as follows:

| | Average µg per ml | Average µg per ml 3 days after last dose |
|-----------------------|-------------------|---|
| After 0.5 G 6 hourly | 790 | 48 |
| After 0.75 G 6 hourly | 1,138 | 5 |

Thus the bulk of the drug is excreted, so that if toxic effects are produced they should soon be relieved by discontinuance of therapy.

Excretion in the faeces This is small in amount and not significant

Presence in body fluids Approximately the same concentrations are found in the pleural and cerebrospinal fluid as are found simultaneously in the blood

CLINICAL TRIALS

The results of trials in tuberculosis are described in Volume II of this series. Apart from these, the main field of inquiry has been in urinary tract infections. Herrold, Boand, and Kamp (1955) treated 124 patients who had been previously treated with various other drugs with only temporary success. The majority of these suffered from some obstruction to their urinary flow, such as an enlarged or infected prostate. The infections were mainly from *E. coli*, proteus, *Aerobacter aerogenes*, pseudomonas, paracolon bacilli or staphylococci and streptococci. The patients were given 0.5 to 1 G daily by mouth when the infections were chronic, but patients with gonorrhoea received 0.25 G 4 times a day together with streptomycin in some cases. As a result, of 49 patients with bladder and upper urinary tract infections, 24, or 49 per cent, were improved or cured, of 19 patients with lower urinary tract infections 10, or 53 per cent, were improved or cured, of 51 gonococcal infections in males 3 or 6 per cent, were improved or cured, and of 5 patients with non specific urethritis, all were found to have improved or recovered. The side reactions with this dose were few: only 2 occurred in 51 patients with gonorrhoea. These reactions consisted of dizziness, a feeling of weakness, vertigo, drowsiness, light headedness and ocular disturbances. One patient suffered from a gastro intestinal disturbance. Medication was stopped in 1 case owing to apparent hypersensitivity, but as a rule mild reactions tended to disappear with the continuation of treatment. Children tolerated the antibiotic well: it was tolerated worst by the elderly. Herrold *et al* (1955) came to the conclusion that the most suitable dose was 1 G daily given in divided doses. They emphasized, however, that the results of treatment often appeared only 10 to 14 days after treatment was administered. Relapse was common: however unless improvement was accompanied by disappearance of the offending bacteria. Little evidence was found of the development of resistant organisms during treatment. Robinson *et al* (1955) reported on the treatment of 22 patients 3 of whom had pyelonephritis. The dose used was 0.8 G daily in divided doses. Two of the 3 patients gradually responded to treatment. Lillick, Strang, Boyd, Schwimmer, and Mulinos (1956) obtained good responses in 6 of their 7 cases of urinary tract infections, and Marmell and Prigot (1956 *b*) who treated cases of gonorrhoea with the dose used by Herrold *et al* (1955) observed little response, although over 1 G daily was given.

Other diseases which were treated were as follows

Respiratory Infections

Seventeen cases of pneumonia were treated by Robinson *et al* (1955). In only 3 was a good response obtained but gradual improvement occurred in 6. This is in marked contrast to the findings of Lillick *et al* (1956) who observed good responses in 38 out of 45 cases with respiratory infections.

Herpes

Lillick *et al* (1956) claimed good responses as a result of treatment in all of 14 cases

Venereal diseases

Apart from gonorrhoea, in which the response was poor, Marmell and Prigot (1956 *b*) used cycloserine in 2 cases of lymphogranuloma venereum and 2 of granuloma inguinale. There was no noticeable improvement in either case of lymphogranuloma venereum after total doses of 10 and 21 G respectively, but with granuloma inguinale the lesions progressed favourably until they had healed by the time 20 or 30 G had been administered

Miscellaneous conditions

Cycloserine was given a trial in such conditions as infectious mononucleosis, infective hepatitis, and pyrexia of unknown origin by Lillick *et al* (1956). None of these conditions showed any response except for 2 cases with pyrexia of unknown origin in which the fever subsided

On studying these somewhat unsatisfactory results, it is as well to remember the observed phenomena of synergism between cycloserine and other antibiotics. Indeed, Robinson *et al* (1956) observed in man that small doses seemed to potentiate the action of penicillin or dihydrostreptomycin. Nevertheless, the relative ease with which toxic effects can be produced in man do not encourage the use of this antibiotic unless others available have failed to have a therapeutic effect

CHAPTER 5

ANTIBIOTICS OF LIMITED CLINICAL APPLICATION OWING TO SOME TOXIC EFFECT

TYROTHRICIN, GRAMICIDIN S, BACITRACIN, POLYMYXIN, NEOMYCIN

TYROTHRICIN AND ITS DERIVATIVES

GENERAL CONSIDERATIONS

This antibiotic was prepared even before penicillin. Dubos in 1939 described an extract he had made from a spore bearing bacillus, which was later found to conform to the characteristics of *Bacillus brevis* (Dubos and Hotchkiss, 1941). The extract made from the culture both killed and lysed pneumococci, streptococci, and staphylococci when incubated with these organisms at 37° C. As with many other antibiotics the extract was stable in alkaline solution but was inactivated at a pH lower than 5.5. Work on this extract led to the separation of tyrothricin into 2 component substances, gramicidin and tyrocidine (Hotchkiss and Dubos, 1940), the former having the greater antibacterial activity.

TABLE 9 ANTIBACTERIAL ACTIVITY OF GRAMICIDIN

| Organism | mg per ml of gramicidin in broth containing 1 per cent serum albumin | by tissue culture method, µg per ml |
|---|--|--|
| <i>Str. pneumoniae</i> | 0.005-0.02 | 0.5-2.5 |
| <i>Str. pyogenes</i> (Groups A, B, and C) | 0.01-0.05 | 5.0-20.0 |
| <i>Str. pyogenes</i> (Group D)* | 0.05-0.2 | |
| Anaerobic streptococcus | 0.01-0.02 | 20-60 |
| <i>Lactobacillus</i> | 0.05-0.5 | .. |
| <i>Staph. aureus</i> | 0.5-5.0 | 100-> 300 |
| <i>C. diphtheriae</i> and diphtheroid bacilli | 0.05-5.0 | . |
| <i>Myc. tuberculosis</i> | 0.02-0.05 | . |
| <i>B. anthracis</i> | 5.0-20.0 | . |
| Saprophytic aerobic sporulating bacilli | 0.1-1.0 | .. |
| <i>Cl. tetani</i> and other sporulating bacilli | 0.02-0.5 | .. |
| <i>Neisseria gonorrhoeae</i> | 0.5-10.0 | .. |
| <i>Neisseria meningitidis</i> | 0.5-10.0 | . |

* Including enterococcus and *Str. faecalis*

From the data of Dubos quoted by Henleerson (1946) and from Herrell and Heilman (1941)

Further work on gramicidin examined the extent of its antibacterial activity *in vitro*. Table 9 gives some idea of the antibacterial range of gramicidin, but methods for testing the sensitivity of organisms to antibiotics had not then

been standardized as they are at present so that results are not strictly comparable. Gram negative bacteria were not inhibited nor were any of these organisms lysed. Tyrocidine was found to have only one tenth to one fiftieth the activity of gramicidin against Gram positive cocci, but to be somewhat more active against Gram negative cocci and bacilli. Tyrocidine also appeared to be bactericidal as well as bacteriostatic (Dawson, Hobby, Meyer, and Chaffee, 1943, Downs, 1942, 1943, Dubos and Hotchkiss, 1941, Herrell and Heilman, 1941, Hobby, Meyer, and Chaffee, 1942, Robinson and Graessle, 1942, Tishler, Stokes, Trenner, and Conn, 1941, Waksman and Woodruff, 1942).

Acquired resistance

Rammelkamp (1942 a) showed that increased resistance to tyrothricin could be induced both *in vitro* and *in vivo*, and these findings were confirmed by Philips and Barnes (1942) and by Hotchkiss (1944). From the clinical point of view it is of interest to note the observations of Lund and Swanton (1954) who studied the incidence of organisms resistant to 20 μg of tyrothricin per ml in children who used a dentifrice containing tyrothricin 2 to 3 times daily for 2 years. At the end of this time swabs were taken from their gums and cultured. Although streptococci, pneumococci, coryne bacteria, neisseria, and micrococci were isolated from the swabs, all of these organisms were inhibited by 3 125 μg of tyrothricin per ml and there was no significant difference in the sensitivity of streptococci and corynebacteria grown from the gums of these children and those of 107 other children who had had no tyrothricin in their dentifrice.

Toxicity

Unfortunately both these substances were found to haemolyse erythrocytes (Heilman and Herrell, 1941, Rammelkamp and Weinstein, 1941) Dubos and Hotchkiss (1941), under the conditions of their experiment found tyrocidine to be haemolytic but not gramicidin. This was not fully confirmed by Mann, Heilman and Herrell (1943) who found that 5 per cent horse serum in the medium eliminated the haemolytic activity of tyrothricin and greatly reduced that of gramicidin. Leucocytes and tissue cultures were also unfavourably influenced by both gramicidin and tyrocidine (Rammelkamp and Weinstein 1942, Dubos and Hotchkiss, 1942-3). At a later date Krupin (1951) claimed that this haemolytic action could be counteracted by growing *B. subtilis* in the test media containing gramicidin.

When gramicidin was given by mouth to mice and rats these animals could tolerate as much as 1 G per kg (Robinson and Molitor, 1942), but this may have been due to destruction of the antibiotic in the gastro intestinal tract. Intraperitoneal injection of 60 mg per kg was fatal to mice in 7 days, but the animals survived a dose of 10 mg per kg. Intravenous injection was fatal in 7 days at a much lower dose, 3.75 mg per kg, while higher doses killed within a day. When dogs were used, intravenous injection of only 2 mg per kg was fatal, according to Rammelkamp and Weinstein (1942). These workers reported extensive liver and kidney damage after administration of the antibiotic. Tyrocidine was equally harmless when given by mouth,

but of the same order of toxicity by intraperitoneal and intravenous injections in mice and rats

Tyrothricin, being a variable mixture of the two components, also varied somewhat in its toxic effects. Daily intravenous injection of 0.4 mg per kg of an early preparation killed 7 out of 8 dogs according to Macleod, Mirick, and Curnen (1940). If the dose was lowered to 0.3 mg per kg petechial haemorrhages and necroses were found in the liver and spleen, kidneys, heart, and lungs, but with 0.2 mg per kg only minor changes were seen. Rammelkamp and Weinstein (1942) confirmed the deleterious effects of intravenous injection of tyrothricin in dogs and also found that intradermal injections or injections into body cavities were toxic. In man, however, 200 mg of the drug could be injected without ill effect into the pleural cavity when this was infected and covered with granulations and fibrin. At a later date the usual complications of antibiotic therapy in man were sought. Grolnick (1946) examined the possibility of sensitizing patients by applications of tyrothricin but could find no evidence of this. Strips of filter paper soaked in 20 times the concentration of tyrothricin required for therapeutic effect were placed for 48 hours on the arms of 171 men and women and the effect noted. Nevertheless Kozoll, Meyer, Hoffman, and Levine (1946) and Goldman, Feldman, and Altemeier (1948) observed an eczematoid dermatitis around lesions to which tyrothricin had been applied. In the patient described by Goldman *et al* (1948) patch tests were carried out after the dermatitis had subsided, and revealed the patients' hypersensitivity to various agents including tyrothricin, penicillin, and bacitracin. A direct effect on mucous membrane was observed by Seydell and McKnight (1948). In these patients tyrothricin had been used in a concentration of 1:5,000 as an intranasal spray or as nasal drops. Immediately or very shortly after treatment patients lost their sense of smell or found it had become abnormal. The condition persisted for 4 to 8 months.

Administration

From experiments in animals it was found that tyrothricin and its components were therapeutically inactive when administered parenterally (Robinson and Graessle, 1942). This is perhaps because the drug is inactivated by serum (Rammelkamp and Weinstein, 1941). However, in confirmation of Dubos's work, these workers were able to protect mice from 10,000 lethal doses of streptococci injected by the same route, by injecting 8 to 16 μ g of either tyrothricin or gramicidin intraperitoneally. Protection was not afforded against *Staph aureus* or *Cl welchii*. With similar treatment Tillett, Cambier, and Harris (1943) protected mice against pneumococcal infection, and Hoogerheide (1940) and McDonald (1940) against anthrax. These experiments serve to support the conclusion that if the toxic effect of the drug is to be avoided administration should be confined to the oral route and to local applications to infected surfaces.

In spite of the serious toxic effects of intravenous injection of the drug in experimental animals, Biro, Szekeley, Votán, Nagy, Endes, and Bálint (1950) prepared a solution for intravenous use which could be infused into rabbits for 2 weeks without any evidence of damage to the kidneys, liver, spleen, lungs, or bone marrow. The preparation was then given to 16 subjects

without producing abnormalities in tests done on the urine, blood, and liver function. These authors thus felt justified in treating 73 cases, and 85 per cent of these recovered from their various infections.

As far as oral administration was concerned there seemed little hope of the drug being therapeutically effective after the experiments of Weinstein and Rammelkamp (1941). These authors found that when mice were fed a susceptible Gram positive organism—*Lactobacillus acidophilus*—in a diet which encourages the growth of this organism, the incidence of lactobacilli in the stool flora was unaffected by the administration of 0.5 mg of tyrothricin daily or twice daily for over 3 weeks. In both the treated animals and the controls lactobacilli continued to constitute as much as 90 per cent of viable organisms. These results were attributed to the destruction of tyrothricin by pancreatic or intestinal juice. Rodaniche and Palmer (1943), however, produced a considerable reduction in the number of faecal streptococci by administering tyrothricin after the numbers of coliform organisms had been reduced to a minimum by means of a diet containing 3 per cent succinylsulphathiazole.

The drug was most effective when given as a local application and even then its use had to be limited to wounds where granulations were plentiful (Howes 1946b). Solutions of tyrothricin were made up in alcohol and glycerin and diluted so as to form colloidal suspensions in water, so that the final concentration of the drug was 0.5 mg per ml. With a small amount of cationic detergent both gramicidin and tyrothricin could be completely dispersed in water (Dubos, personal communication). A solution of the drug in 95 per cent alcohol was applied to ulcers by Rammelkamp (1942b) and other preparations were made from solutions in propylene glycol and carbowaxes. A powder, diluted with boric acid, was used for insufflation onto infected areas.

CLINICAL TRIALS

Infected ulcers, wounds, and burns

In infections due to *Str. pyogenes* very good results followed the application of tyrothricin or gramicidin. The lesions frequently became free from streptococci after treatment and healing took place or grafting was made possible. Such results were reported by Herrell and Heilman (1941), Herrell (1943), Rammelkamp and Keefer (1941), Rammelkamp (1942b), Wright, V. W. M. (1942), Kvale, Barker, and Herrell (1944), Kozoll, Meyer, Hoffman, and Levine (1946), Lask (1948), Merrill (1948), and Mom (1946). It is interesting to note that, as was the experience with penicillin, Kozoll *et al.* (1946) noticed that in wounds tyrothricin seemed to encourage the growth of *Ps. pyocyanea* even though there was no evidence of this *in vitro*.

Respiratory tract infections

Rammelkamp (1942b) used tyrothricin for the treatment of pharyngitis, and Schoenhach, Enders, and Mueller (1941) claimed that carriers could be cleared of streptococci by the use of this drug. Lindsay and Judd (1943), however, could not demonstrate any beneficial effects from the use of

tyrothricin in their patients, and the results of other workers for example Crowe, Fisher, Ward, and Foley (1943) and Herrell (1945) were not conclusive.

Otolaryngological Infections

Tyrothricin was used by Rammelkamp (1942 *b*) in repeated dressings after mastoidectomy operations. When streptococci were present they often disappeared with this treatment. Bordley, Crowe, Dolowitz, and Pickrell (1942) and Crowe and Ward (1943) also used tyrothricin in otolaryngological work. Although they were not able to give exact results from their work, they were convinced that when gramicidin or tyrothricin was used to paint an area of operation or to pack the cavity left behind after operation they were invaluable in reducing the post operative discomfort usually suffered by patients with septic infections due to streptococci or pneumococci. These workers emphasized the fact that when staphylococci were present these organisms were uninfluenced by the antibiotic. It was perhaps for this reason that Crowe (1944) advocated the packing of cavities after operation with penicillin, reserving tyrothricin for painting the wound immediately after operation. It is in this field that tyrothricin has been used most frequently, for the small cavities about the nose and ear lend themselves to local applications better than most other parts of the body. The drug has been used in otorhinolaryngological conditions by Falbe Hansen (1949), Virsult Kretschmar (1948), by Muller (1948) who washed out infected maxillary sinuses twice daily with a solution containing 0.3 mg per ml but without great success and by Sambataro and Clerici (1950) who instilled the drug intranasally in patients suffering from common cold, nasal allergy, or catarrhal sinusitis.

Infections of the eyes

Tyrothricin was usually used in concentrations of 1:5000 for instillation into the conjunctival sac but Herrell (1945) used a concentration of 1:200 in a few cases without detriment to the tissues of the eyes. Without determining the nature of the causal bacteria it is of course not possible to forecast what effect the drug would have. Nevertheless Streicher (1944) met with some success in the treatment of conjunctivitis and keratitis, and Bellows (1943), Heath (1944) and Volner and Cooper (1944) saw some improvement in cases of epidemic keratoconjunctivitis, blepharitis and punctate keratitis associated with chronic conjunctivitis and dacryocystitis. Osterberg (1949) using a proprietary preparation of tyrothricin *Tyrosulin*, had remarkable success in 131 out of 146 cases of blepharitis who were cured in 2 to 3 weeks. He was equally successful in the treatment of acute conjunctivitis and acute superficial keratitis. What is more, he saw no sensitization phenomena in any of his patients. Tyrothricin was also used in an ointment base at a concentration of 1:5000 by Bloomfield (1944) for the treatment of marginal ulcers of the cornea. Four cases, which had resisted previous treatments, responded satisfactorily to this.

Trachoma Bietti (1948) used solutions at concentrations of up to 50 mg of tyrothricin per 100 ml and observed a reduction in the conjunctival flora but no effect on the trachomatous lesions.

Empyema

Irrigation of empyema cavities with tyrothricin solutions after drainage was carried out by Rammelkamp (1942 *b*) and resulted in the rapid disappearance of streptococci from the lesions. Rammelkamp used as much as 100 mg in each instillation. Donaldson and Samuel (1946) were not so fortunate with the cases of empyema which they treated in tuberculous patients. None of these patients had open drainage, and they thus had less chance of ridding the empyemata of their Gram positive cocci. Although these authors did not use more than 10 to 50 mg per instillation, the temperature rose in all patients following the procedure, but the character and amount of the exudate did not alter.

Infections of the skin

Rammelkamp (1942 *a*) again seems to have been the first in this field. He found that skin ailments caused by *St. pyogenes* responded occasionally to tyrothricin, and similar results were reported by Herrell (1945). MacKee, Sulzberger, Herrmann, and Karp (1946) found that when tyrothricin was incorporated in a special base it was useful for controlling the secondary infections superimposed on various dermatoses. These authors were even impressed by the value of the drug in 112 patients with acne vulgaris. The prescription used was as follows:

| | |
|---------------------------------------|--------|
| Sodium mixed alkyl benzene sulphonate | 1.0 G |
| Tyrothricin | 0.1 G |
| Propylene glycol | 10.0 G |
| Distilled water | 88.0 G |

Contrary to the chemical findings of Bloomfield (1944), H. E. Anderson (1946) found that tyrothricin was ineffective when incorporated in the common ointment bases, but obtained good results in three quarters of his 20 cases when wet dressings containing the drug were applied to infected areas. An ointment was again used by Lubowe (1951) but this contained bacitracin as well as tyrothricin. Impetigo, ecthyma, and infected wounds all improved, but ulcers of the feet were more variable in their response, as also were cases of herpes zoster, sycosis barbae, and paronychia. Lubowe had no success in treating folliculitis or hydradenitis suppurativa.

Infection of the bladder

Herrell and Heilman (1941) reported an excellent result in a case of streptococcal cystitis which had been refractory to previous treatment. Solutions of 500 µg per ml were used to irrigate the bladder.

Gonorrhoea

At a time when chemotherapy for gonorrhoea was not particularly satisfactory local irrigations of the urethra for the acute infection were practised. Dub (1944) used this method with tyrothricin and claimed some success.

Infections of the central nervous system

Brain abscesses. Kuzmenko (1949) instilled gramicidin in 12 cases of brain abscess in frontal, parietal, or occipital areas, after the contents of the

abscesses had been aspirated. In 7 other cases the cavities were irrigated every 24 hours after they had been opened. The somewhat drastic concentration of about 1:100 was used. The infecting organisms were streptococci, staphylococci or mixed flora including Gram negative rods. All cases recovered, and suppuration sometimes ceased after a single irrigation. A single irrigation was obviously not reliable, as recurrence appeared in 1 case 16 days after the irrigation. However, 8 of the 12 cases required no more than 2 to 4 aspirations and instillations. In the 7 cases with abscess cavities which had already been opened recovery took longer, and the post operative period was apt to be complicated by prolapse of the brain tissue and growth of Gram negative rods. Kuzmenko (1949) recognized that the open method of treatment had the disadvantage of allowing added and perhaps resistant infection to supervene. Penicillin was also administered to these cases in doses of 100 000 Units intramuscularly every 24 hours, so that the efficacy of gramicidin can hardly be assessed.

Gastro-Intestinal Infections

Tyrothricin was applied by Cantor (1946) to infections involving the rectum. He applied continuous wet dressings post operatively to all rectal and open pilonidal surgery, that is for haemorrhoidectomy, prolapse, excision of anal ulcers, excision and saucerization of fistulae, &c, until healing was completed. The solution of tyrothricin contained 25 mg per ml and this was diluted 50 times in distilled water before soaking the dressings in it. No suppuration of the wounds was seen and healing was rapid, while patients were up and walking within 24 hours of operation. The only disadvantage of this method of treatment seems to have been perianal dermatitis, which sometimes appeared where the wet dressings overlapped the open wound. This however, disappeared rapidly soon after the applications ceased.

Obstetric and gynaecological conditions

Vaginitis Five patients with acute pruritus vulvae and leucorrhoea were treated by Sanchez Iñáñez (1951). In all these cases *Trichomonas vaginalis* was isolated from the vagina and in 1 case there was also a gonococcus and in another a staphylococcus. Tyrothricin was given in a suspension containing 50 mg together with 50 mg of boric acid in 100 G of water. This preparation was insufflated after a douche with 5 per cent lactic acid, every 2nd day for at least 4 applications. After the first application bacteriological examination of vaginal smears was negative in all cases and remained negative for the next 3 months.

Puerperal mastitis Twenty patients with puerperal mastitis due to *Staph aureus* were treated by Schweigmann (1953). An aqueous suspension of tyrothricin was instilled into the abscess cavity immediately after incision of the infected breast and wet compresses were subsequently applied. Schweigmann considered that this method of treatment reduced by half the period for which patients treated with penicillin and the sulphonamides alone were usually kept in hospital.

Diphtheria carriers

Since *C. diphtheriae* was known to be comparatively sensitive to gramicidin,

carriers attending the Infectious Diseases Hospital, Turin, had their throats sprayed with 3 ml of a 1:2,000 suspension of tyrothricin twice a day. After 4 days of this treatment these organisms had disappeared from 98 out of 100 throat swabs. The remaining 2 were refractory to treatment (Baglione and Di Nola, 1950). These workers considered that healthy carriers were slower to react to this treatment than convalescent patients.

The advent of other antibiotics, which can be administered systemically against infections due to Gram positive bacteria, since they have minimal toxic effects, has tended to relegate tyrothricin and its derivatives to the background. Used discreetly in concentrations not exceeding 1:200, however, its bactericidal activity and small power of inducing sensitization in the patient should still give it a useful place in the list of antibacterial agents applicable to man.

GRAMICIDIN S

This antibiotic was isolated by Gause and Brazhnikova (1944). After testing many hundreds of sporulating bacilli from Russian soils in the hope of finding an organism which would antagonize the staphylococcus, they found one which was capable of doing this and from it gramicidin S was isolated and later crystallized. Like gramicidin, it was a polypeptide, being insoluble in water but easily so in alcohol. Its antibacterial activity *in vitro* was promising since it inhibited 18 strains of *Staph. aureus* and 10 strains of streptococci, at a concentration of 1:100,000. Clostridia were even more sensitive, *Cl. welchii* and *Cl. histolyticum* being inhibited by 1:140,000. It was also active in higher concentrations against salmonellae, shigellae, *V. cholerae*, proteus and *E. coli*. Lipoid substances reduced its activity against Gram negative organisms, but did not do so against Gram positive organisms. There did not, however, appear to be any investigation into the influence of blood or serum on the activity of this agent. Its toxicity in white rats was much the same as that of tyrothricin, the LD₅₀ being 17 mg per kg. Its use in man, therefore, was confined to local applications. When used in this way in wounds, solutions of the drug did not produce any damage to the tissues.

Clinical trials

Gramicidin S was used by Sergiev (1944) in 1,500 cases. It was dispensed as a 4 per cent solution in alcohol and then diluted 100 times with water so as to make a final concentration of 0.4 mg per ml. It was also used as an ointment incorporated in castor oil. Sergiev (1944) considered that it was successful in arresting or limiting suppuration in soft tissues: osteomyelitis, empyema, peritonitis, and various skin infections. Lavrentyeva (1947) confirmed its value in the local treatment of empyema. Due to its greater activity against Gram negative organisms it was considered that this substance would be of more value than gramicidin for local application in pyogenic lesions where the causative organisms were not known.

BACITRACIN

GENERAL CONSIDERATIONS

This antibiotic was first prepared by Johnson, Anker, and Meloney (1945) from a culture fluid in which a bacillus obtained from a compound fracture of the tibia had grown. This bacillus was selected because it was particularly active in preventing the growth of colonies of other bacteria in a culture plate made from the wound. Johnson *et al* (1945) considered that the organism was *Bacillus subtilis* but it was later identified as *Bacillus licheniformis* (K. Burden, quoted by Meloney and Johnson, 1949a). Thus it was shown to be the same species of bacterium as that which had produced *Ayfirm* (Newton and Abraham, 1950). Study of the latter substance has led to further information being obtained about the nature of bacitracin (Shirp, Arriagada, Newton, and Abraham, 1949).

Johnson *et al* (1945) defined the Unit of activity of bacitracin in terms of a group A haemolytic streptococcus. A Unit was the amount required to inhibit completely an overnight broth culture of this particular strain diluted in beef infusion broth to 1:1,024. It therefore has no relation to the Unit used for assaying penicillin. Meloney and Johnson (1953a) found that 1 μ g contained between 35 and 50 Units of the active substance. Eventually, an international standard for bacitracin was set up by the World Health Organization (1954). Assays from 6 laboratories in 5 different countries established that 1 Unit contained 0.0182 mg of the standard batch. In other words, 1 mg contained 55 International Units. Bacitracin was found to be readily soluble in water. It could be kept in acid solution at 5° C but not in alkaline solution when the pH was above 9.0. Moreover the antibiotic resisted digestion with both pepsin and trypsin. It was not thought by Anker, Johnson, Goldberg, and Meloney (1948) to be a polypeptide, but unfortunately these authors were not able to obtain the material in a pure state before their first publication and it was thus impossible to attribute unequivocally to bacitracin the various effects produced by their compound in experimental trials. When Craig, Gregory, and Barry (1949) examined the material further they considered that it might be a mixture of polypeptides. Further purification led to the separation of bacitracin into 3 peptides: bacitracin A, B, and C (Newton and Abraham, 1950). Bacitracin B was the least potent antibacterial constituent and also the least toxic, whereas C had a greater acute toxicity than either A or B. The antibacterial properties and acute toxicity tests appeared to run parallel with one another (Newton, Abraham, Florey, Smith, and Ross, 1951). Another constituent, bacitracin F, derived from A, had no antibacterial action against the test organism but was just as toxic as bacitracin A (Coddington, 1954-5). It should therefore be removed from preparations before these are used for systemic injection.

Antibacterial activity

The sensitivity of various organisms to bacitracin *in vitro* is given in Table 10. These organisms were mainly isolated from pathological material. There was not much evidence of cross resistance between bacitracin and

other antibiotics in the streptococcus and the staphylococcus some strains were sensitive to penicillin and bacitracin and chlortetracycline, whereas others were inhibited by one antibiotic and not by the others (Meleney and Johnson, 1947, Meleney, 1950) Pune (1951), however, considered that he had demonstrated cross resistance between bacitracin and penicillin. Evidence *in vitro* was put forward by Bachman (1949) that a synergistic effect was produced by bacitracin and penicillin with certain strains of streptococci and with an organism isolated from the vagina (Goldin and Auerbach, 1950). A synergistic effect was produced by bacitracin, tyrothricin, and cobalt, according to Forni and Ruggerini (1954), and by bacitracin and neomycin, according to Young, Yoshimura, and Felsenfeld (1950). Eagle and Fleischman (1948a) showed that penicillin and bacitracin were also synergistic in the treatment of rabbit syphilis. Synergism was demonstrated between bacitracin and penicillin in strains of staphylococci isolated from 3 cases of osteomyelitis (Meleney, 1957).

TABLE 10 ANTIBACTERIAL ACTIVITY OF BACITRACIN AGAINST ORGANISMS PATHOGENIC TO MAN

| Organism | Unit* per ml causing inhibition |
|---|---------------------------------|
| Haemolytic streptococci | |
| Groups A, B, C, F, G | 0.025-0.003 |
| Group D | 3.0-0.003 |
| Non haemolytic streptococcus | 3.0-0.025 |
| Pneumococcus | 0.1-0.002 |
| Staphylococcus (coagulase positive) | 5.0-0.05 |
| Other micrococci | 5.0-0.003 |
| <i>C. diphtheriae</i> | 0.015-0.004 |
| Meningococcus | 0.01 |
| Gonococcus | 0.006 |
| <i>H. influenzae</i> type B | 0.63 |
| <i>B. anthracis</i> | 0.5-12.5 |
| Anaerobic bacteria | |
| <i>Cl. welchii</i> | 0.025-0.002 |
| <i>Cl. septicum</i> | 0.01-0.002 |
| <i>Cl. sordellii</i> | 0.01-0.003 |
| <i>Cl. novyi</i> | 0.01 |
| <i>Cl. tetani</i> | 0.01-0.006 |
| <i>Cl. histolyticum</i> | 0.025-0.004 |
| Non haemolytic streptococcus | 0.1-0.005 |
| Haemolytic streptococci | 0.01-0.001 |
| Micrococcus | 0.5-0.003 |
| Diphtheroid bacillus | 0.003 |
| <i>Actinomyces israeli</i> | 0.075-0.005 |
| <i>Trep. pallidum</i> (Reiter strain)† | 0.004 |
| <i>E. coli</i> , <i>A. aerogenes</i> , <i>A. cloacae</i> , <i>Proteus</i> , <i>Ps. aeruginosa</i> , } All inhibited by 50 Units <i>B. alkaligenes</i> , <i>Salm. typhosa</i> , <i>Sh. alkalescens</i> and fungi } per ml or more | |

* 1 Unit = approximately 0.0182 mg of a standard batch of bacitracin (Humphrey, Lightbown, Mussett and Perry, 1953)

† From the data of Meleney and Johnson (1949a) and Eagle, Musselman, and Fleischman (1948)

Acquired resistance

Four strains of *Staph. aureus* were studied by Stone (1949). He found that resistance of the staphylococci to bacitracin could be induced, but

that it was slow and irregular in appearing. Moreover the resistance could be lost by daily subculture in bacitracin free broth. Unlike Paine (1951), Stone did not find any increase in the resistance of the organisms to penicillin as their resistance to bacitracin rose, or *vice versa*. No staphylococci containing an enzyme which destroyed bacitracin were found (Meleney, Altemeier, Longacre, Pulaski, and Zintel, 1948). Gezon, Tasan, and Collins (1950) could not find any evidence that β haemolytic streptococci developed any resistance to penicillin during a course of treatment with bacitracin.

Toxicity

Meleney and Johnson (1955) described 3 periods during which the toxicity of commercial bacitracin differed. During the earliest phase patients were treated with bacitracin obtained from the surface growth of cultures and toxic effects were slight (Longacre and Waters 1951 *b*, Reisner, Bailey, and Appelbaum, 1951). When the antibiotic was produced by the deep tank method there were disturbing evidences of nephrotoxicity (Zintel, Ma, Nichols, and Ellis, 1949; Michie, Zintel, Ravdin, and Ragni, 1949). Lethargy was also observed in the patients treated by Pulaski and Connell (1949 *a*). When the specifications of the Food and Drug Administration had to be met, namely that preparations had to have an LD_{50} of no less than 500 Units for a 20 g mouse, the toxicity of the material was kept within measurable limits. The minimum requirements were still further raised at a later date, each batch for systemic use had to contain 35 to 50 Units per mg and it was stipulated that a dose of 100 Units of such a preparation administered intravenously to a series of 20 g mice was not to produce death in any of the animals.

It is somewhat difficult to disentangle the toxic effects due to inadequate purification from those which persist after the purification of the material. It was, however, considered by Newton *et al* (1951) that the nephrotoxicity of commercial preparations was due at least in part to the active antibacterial constituents of bacitracin and that therefore there was little prospect of isolating a constituent which was both potent antibacterially and also non toxic.

With these experimental conclusions in mind, it seems well to mention the evidence about the toxicity of the drug which has accumulated since bacitracin was first isolated. Scudl and his collaborators carried out a number of experiments in which the qualities of earlier samples were tested. Scudl and Antopol (1947) tested 4 samples in mice and rats. Tubular necrosis in the kidneys of mice was found after large doses, but the changes in rats were not so severe. The LD_{50} seemed to be independent of the presence or absence of the antibacterial factor. The method of administration affected the toxicity: intravenous injection produced more lethal effects than intraperitoneal, subcutaneous, or oral administration. Mice could live for 5 days after subcutaneous injections of up to 50 Units and rats could survive a similar dose for a fortnight. Such toxic effects were not seen in dogs (Scudl, Clift, and Krueger 1947). In monkeys, however, 1 500 Units per kg given intramuscularly twice a day over 45 days produced both sugar and albumin in the urine during the course of treatment (Scudl, Coret, and Antopol, 1947).

These workers concluded that large doses, for example the LD_{50} , produced damage to the renal tubules and tubular necrosis in the mouse, and to a much smaller extent in the monkey. The rat and dog, however, were relatively immune. These authors also found that the commercial samples used in their experiments varied in toxicity but not in accordance with their antibacterial activity. Smith, Schultz, Ott, and Payne (1949) confirmed the effect described by Seudt and Antopol (1947) in the tubular epithelium of mice and they did not find any greater damage from lots produced by deep cultures than from lots from surface cultures. In experiments on the toxicity of bacitracin by oral administration, Payne, Spencer, and Schultz (1951) found that they could give as much as 125,000 Units per kg to mice daily for 30 days and up to 25,000 Units per kg to dogs for the same time without any cumulative evidence of toxicity. This might be explained by the poor absorption of the antibiotic from the gastro intestinal canal.

When the antibiotic was applied to man the first 100 cases were treated by local applications (Meleney and Johnson, 1947) and no signs of toxicity appeared. When, however, the antibiotic was given by intramuscular injection in doses of 10,000 to 100,000 Units 4- to 6 hourly there was evidence of kidney damage (Meleney and Johnson, 1949 b) and the toxicity was observed to vary with different lots. Intravenous administration over 1 hour of 23,600 Units of some commercial batches produced no significant changes in glomerular filtration rate, maximal tubular excretion of para aminohippurate, or maximal tubular reabsorption of phosphate (Michie *et al*, 1949). In spite of this, however, there was a gradual decrease during the experiment in the average values of maximal tubular excretion of para aminohippurate, which suggested some tubular damage. When bacitracin was continued in doses of 49,000 to 50,000 Units 6 hourly by intramuscular injection, moderate to severe renal damage followed. Administration of the drug for 2 days and again for 1 day showed 6 days later that there had been marked impairment in the tubular excretion of para aminohippurate but no significant diminution in the remaining renal functions. Zintel *et al* (1949) also observed local pain and petechiae round the site of injection, skin rashes, nausea and vomiting, albuminuria, ringing in the ears, and a peculiar taste in the mouth, in a fair proportion of patients treated with 50,000 Units 6 hourly by intramuscular injection. With the lower doses of 10,000 to 20,000 Units 6 hourly Pulaski and Connell (1949 a) still found that injection inflicted pain, and anorexia and nausea developed after a total dose of 150,000 Units had been given. Urinary frequency and lumbar pain lasting for 3 weeks to 3 months followed total doses of 275,000 Units. Pulaski and Connell also found that phenolsulphonphthalein clearance was delayed and that non protein nitrogen levels in the blood rose. Proteinuria and sporadic glycosuria lasted for up to 11 days after treatment ceased. With still lower doses of 200 to 400 Units per kg 6- to 12 hourly for 7 days, or even with 1,500 Units per kg once daily for 2 days, Miller, McDonald, and Shock (1950) found that proteinuria developed in all of 148 patients with syphilis, irrespective of the batch of antibiotic used or of the size of the dose within these limits. Miller *et al* (1950) tested the effect of the antibiotic on 12 males without any signs of cardiovascular, renal, or infectious disease. Inulin and sodium para-aminohippurate clearance were both reduced, so that it was inferred that both glomerular filtration and tubular excretion were depressed. There was

a slow return to normal function over 6 to 9 weeks. Persistent glycosuria also appeared during the depression of renal function, but the blood urea nitrogen did not rise significantly nor was there any change in the arterial blood pressure. Eventually Meloney and Johnson (1955) advised that even with the lots which had conformed to the Food and Drug Administration's specifications doses should not exceed 100,000 Units a day or 10,000 to 25,000 Units every 6 to 8 hours.

Animal protection tests

Staphylococcal infection Meningitis produced by inoculating staphylococci into the cisterna magna of dogs was found to be controlled when bacitracin was instilled into the space within 2 to 3 hours of the inoculation (Teng and Meloney, 1949).

Syphilis in rabbits Eagle and Fleischman (1948 *a* and *b*) tested the action, *in vitro*, of bacitracin against the Rexter strain of *Trep pallidum* and also its efficacy in eliminating from experimental lesions a strain known to be pathogenic. It was found that 36 Units of bacitracin per kg given by intramuscular injection to the rabbits eliminated treponemes from chancres. Bacitracin was found to be bactericidal to treponemata (Eagle *et al*, 1948) but much larger doses, however, were required to produce this effect and to produce permanent healing of the chancre. The dosage required for permanent healing was 1,150 Units per kg repeated once daily for 4 days or a single dose of 5,000 Units per kg.

Clostridial infections Sandusky and Keeble (1949) studied the effect of bacitracin on wounds in guinea pigs infected experimentally with *Cl welchii*. They found that, whereas 112 out of 114 control animals died from the infection, 50 out of 51 animals given 3 Units of bacitracin per G weight immediately after inoculation survived the test period of 15 days. It was also found that the effectiveness of the antibiotic diminished if there was more than 2 hours' delay before the bacitracin was administered. After a delay of 6 hours no prophylactic effect was obtained.

Pinworm infection An attempt was made by Wells (1952 *b*) to see if bacitracin would have any effect on pinworm infestation of mice. Helminth-free animals were inoculated with 300 embryonated eggs of *Aspicularis tetraoptera* each and were given 20,000 Units of bacitracin per kg, or other antibiotics, from 2 days before the infestation to 7 days after. Little effect was produced on the number of worms found in the mouse's gut unless treatment was continued for 14 days after inoculation.

Administration

Oral administration

In experimental work on the dog it was early found that little of the drug was found in the blood following administration by mouth. Scudt *et al* (1947 *a*) could find no activity in the blood and no activity in the faeces after 3,000 to 6,000 Units per kg had been administered to the animals by mouth. This fact may readily explain the lack of toxicity of the drug when administered by this route. On the other hand, when the drug was administered by stomach tube, Bond, Vanderbrook, Wiley, and Nook (1948) found detectable blood

levels in the serum and high levels in the urine, the numbers of streptococci and spore forming anaerobes in the faeces being greatly reduced. In man, Longacre and Waters (1951 *a*) confirmed the findings of Scudi *et al* (1947). The contradictory results found by these different workers may have depended on whether the end of the tube used by Bond *et al* (1948) rested in the stomach or in the duodenum. If this is so, then the inference must be that bacitracin is destroyed in the stomach rather than that it is not absorbed by the intestine.

Parenteral administration

Absorption into the blood and tissues from subcutaneous, intramuscular, and intravenous administration was demonstrated in experimental animals by Scudi *et al* (1947 *a*), Teng, Levin, and Meleney (1949), and Howe, Wigglesworth, and Kahn (1953). Only the brain seemed impermeable to the drug. After repeated intramuscular injections of 50,000 Units every 6 hours for several days, Zintel *et al* (1949) found concentrations in the blood of only 1 to 3 Units per ml and levels similar to those in the serum were found in the pleural fluid. A more gradual increase occurred in ascitic fluid but levels here eventually exceeded those obtained simultaneously in the blood. Little penetration into the pericardial or cerebrospinal fluid was observed. Only with injections which would now be considered abnormally high did Teng and Meleney (1950 *a*) detect the presence of bacitracin in the cerebrospinal fluid of patients with meningitis.

The results of intramuscular injection of the drug in both experimental animals (rabbits) and man were studied by Eagle, Newman, Greif, Burkholder, and Goodman (1947). Three different batches of bacitracin were used but when allowance was made for their different degrees of activity the general result remained the same. Using a modified Rammelkamp-Rantz-Kirby method of assay these authors found that levels of bacitracin could be detected in the serum from one quarter of an hour up to 8 hours after intramuscular injection of single doses of 120 to 150 Units per kg of body weight. These concentrations were generally many times those required to inhibit susceptible organisms (see Table 10, p. 105), even an injection of as little as 15 Units per kg would produce a high enough concentration in the serum to inhibit for 4 hours all but the least susceptible strains of streptococci. The figures given by these authors for blood concentrations following a single injection were as follows:

| Dose in Units per kg | No of sub- jects | Units per ml in blood at hours after injection | | | | | | | |
|----------------------------|------------------------|--|---------------|-------|--------|---------|-----------|---------|----|
| | | $\frac{1}{4}$ | $\frac{1}{2}$ | 1 | 2 | 4 | 6 | 8 | 24 |
| 150* | 2 | 0 | 19 | 25-43 | 33-43 | 17-20 | | < 15-30 | |
| 120 | 1 | | 66 | 19 | 23 | 75 | 15 | < | |
| 90 | 1 | 10 | 20 | 27 | 14 | 72 | 23 | 18 | |
| 60 | 1 | 4.5 | 12 | 20 | 29 | 14 | < 15 | | |
| 45 | 2 | 1.8-4.5 | 3.3-7.2 | 6-12 | 3.6-12 | 1.8-3.6 | < 1.5-2.3 | | |
| 20 | 1 | 3 | 3.8 | 3.8 | 2.5 | < | | | |
| 15 | 1 | 1.8 | 3.0 | 3.8 | 3.3 | 1.5 | | | |

* Equivalent to 10 500 Units in a man weighing 70 kg.

From their figures Eagle *et al* (1947) were able to demonstrate that the concentrations in the blood varied fairly consistently as the size of the dose

given Reisner *et al* (1951) recorded the results of injections given at 6 hourly intervals to patients with pneumonia. Although the doses given by these authors were much higher than those of Eagle *et al* (1947) and were repeated, the concentrations found in the blood serum were lower than the latter's figures. Presumably all assays were made at the end of each 6 hour period. The blood levels following these injections were as follows

| <i>6 hourly dose (in Units)</i> | <i>No of estimations</i> | <i>Units per ml in blood serum</i> |
|-------------------------------------|------------------------------|--|
| 99 000 | 2 | 1.3-2.0 |
| 76 000 | 5 | 0.24-2.0 |
| 60 000-66 000 | 3 | 0.09-0.75 |
| 47 000-50 000 | 7 | 0.06-1.0 |
| 38 000 | 2 | 0.35-2.3 |
| 33 000 | 3 | 0.08-1.0 |

From these figures there seems to be little relation between the dose given and the concentrations in the blood serum, unless the explanation is that bacitracin, in doses up to 60,000 Units, disappears from the blood stream within the 6 hour intervals at a rate commensurate with the size of the dose.

When measuring the urinary excretion Eagle *et al* (1947) found that the concentration of bacitracin in the urine remained at a fairly constant level for 2 to 4 hours after an injection. After this time the rate of excretion increased, thus 7, 36, 66, and 87 per cent of the dose had been excreted at 1, 2, 4 and 6 hours after it was injected. From experimental work on rabbits, supported by figures obtained in man, these authors concluded that the low renal clearance approximated the glomerular filtration rate.

Local administration

Meleney and Johnson (1947) first demonstrated the clinical effect of bacitracin given by local administration. They first used crude filtrate, then a partially purified and concentrated product prepared for them by Dr Anker, and eventually a preparation made by a commercial firm. With preparations containing so many variables, application of the drug was confined to solutions and water soluble bases. With these Meleney and Johnson demonstrated the antibacterial effect of either surface application of the drug or its local injection into tissues surrounding infections such as abscesses, furuncles, and carbuncles. Miller, Slatkin, and Johnson (1948), using an ointment base containing 480 Units of bacitracin per G, confirmed the good clinical effects reported by Meleney and Johnson (1947), but also mentioned that contact dermatitis had been seen in a very few cases.

Administration by aerosol

For this method of administering bacitracin Prigal and Furman (1949) used an aerosol made up of 12 000 to 134 000 Units of bacitracin dissolved in 2 ml water and 20 ml propylene glycol. The aerosol was administered by an apparatus consisting of a combined steam generator and aerosolizer. No detectable concentration of the drug was found in the serum after this mode of administration but up to 10 Units per ml were found in the urine. Some absorption therefore must have taken place. Inhalations could be combined with 100 000 Units of penicillin and 0.5 to 1 G of streptomycin.

calcium chloride with added effect. These workers, however, were not able to show any correlation between the change in the bacterial flora of the sputum and the clinical effect of the drug.

Combined administration of bacitracin and other antibiotics

Because the range of its antibacterial action *in vitro* is limited, trials have been made of bacitracin combined with other antibiotics. The synergistic action of bacitracin and penicillin has also been demonstrated *in vitro*. When combined with tyrothricin, neomycin, polymyxin, or one of the tetracyclines, however, it was hoped that bacitracin would act against certain species of bacteria, mainly Gram positive organisms, and the other antibiotic would deal with Gram negative organisms or, in the case of neomycin, even with *Mycobacterium tuberculosis*. Bacitracin combined with neomycin seems to have been used most successfully for lesions susceptible to local administration of the drugs, such as skin infections. In the belief that tyrothricin had some action against Gram negative rods, Lubowe (1951) applied an ointment containing 500 Units of bacitracin and 800 μ g tyrothricin to various skin infections with varying success. Bacitracin and neomycin have been applied together by Gade, Korner, and Sylvest (1953), Montgomery and Montgomery (1953), Perdrup (1953), Rattner and Rodin (1952), and Szabo (1953).

In the treatment of infected wounds, cavities, and other soft tissue lesions, bacitracin and neomycin have been found particularly useful by Holzhausen (1955) and by Klima (1955). The latter author found the combination beneficial for irrigating tuberculous fistulae and empyemata cavities, and as an aerosol for patients with bronchiectasis.

Moritsch (1955) prepared patients for operations on the intestine by administering tablets containing 250 Units of neomycin and 12,500 Units of bacitracin. One or two such tablets were given by mouth 3 times a day for 3 days, together with bowel washouts. The outcome from a bacteriological point of view was, however, equivocal.

Polymyxin has been combined with bacitracin for the treatment of pyoderma. This ointment was used with outstanding success for pyoderma by Pass and Rattner (1954) and in various infected dermatoses by Kile, Rockwell, and Schwarz (1953).

CLINICAL TRIALS

As has been mentioned before, the first clinical trials were confined to cases which could be treated by local applications. In these, direct application or instillation into the tissues surrounding the lesions had a beneficial effect on carbuncles, furuncles, abscesses and also on infected sebaceous cysts, operative wounds, ulcers, and impetigo (Meleney and Johnson 1947). When systemic administration was attempted (Meleney *et al.*, 1948) three quarters of 105 cases with synergistic gangrene, cellulitis, deep abscess, and septic wounds responded to treatment. These were cases in which sulphonamides and penicillin had produced no demonstrable effect, the infecting organisms being *Staphylococcus aureus*, haemolytic and non haemolytic streptococci, and microaerophilic and anaerobic staphylococci. The least

satisfactory results were obtained in the treatment of thrombophlebitis and brain abscess. The good results in soft tissue infections were confirmed by Longacre and Waters (1951 *b*). Because of its efficacy when applied locally to lesions, the clinical use of bacitracin has been largely in the field of surgery, for the drug can be applied at operation when the wound is open or when dressings are applied to open lesions. It is therefore only occasionally that bacitracin has been used to treat infections of a non-surgical nature, even though there is experimental evidence that it could be of value as, for example, in syphilis.

By 1952 Meleney, Johnson, and Teng had collected a further 160 cases, 105 of which were treated by bacitracin alone. Amongst these were surgical infections such as chronic osteomyelitis and gas gangrene as well as other conditions similar to those already described. Non-surgical infections and neurosurgical infections were also included. Favourable results were obtained in 78 per cent of the surgical infections and in a high proportion of the other cases. Meleney *et al* (1952) made the interesting statement that not only did the majority of streptococcal, staphylococcal, and clostridial infections respond well, but cases with a mixture of organisms or with Gram-negative rods also frequently responded. These conclusions had already been reached in 1949 by Meleney, Longacre, Altemeier, Reisner, Pulaski, and Zintel.

Amoebiasis

E. histolytica was found to be susceptible to bacitracin, as were such intestinal bacteria as clostridia and cocci (Faust, 1949). In consequence, clinical trials of the antibiotic in intestinal amoebiasis were begun by Most. Most first described the results in more than 50 patients who received relatively high doses by mouth (Most, 1949). He stated that the clinical response could be dramatic in severe cases, but although 60 to 80 per cent of patients had remained free of entamoeba for 6 months after therapy, in the more severe cases the disappearance of *E. histolytica* was only temporary and limited to the period of treatment. The action of the antibiotic was ascribed to the deterrent effect on the growth of amoebae owing to its control of Gram positive organisms and anaerobes in the stools. This work was elaborated in 1950 (Most, Miller, Grossman, and Conan, 1950 *a* and *b*). The patients in this series showed *E. histolytica* in 2 separate specimens of stools before treatment began. The 51 patients were given 40,000 to 120,000 Units of bacitracin daily by mouth for 5 to 20 days. In 48 of these patients the amoebae disappeared, but in 14 of these they reappeared within a week to a year after treatment, usually within 5 weeks. Nine patients were treated again but of these 6 relapsed. Three of these were treated a 3rd time and 2 relapsed. In spite of this equivocal evidence of the curative power of bacitracin in amoebiasis, ulceration was seen to have healed in 8 severe cases. Most *et al* (1950 *b*) remarked on the simultaneous disappearance of enterococci and clostridia, and discussed the possible relation of these organisms to the pathogenesis of amoebic colitis. As little, if any, bacitracin was absorbed after oral administration, no toxic effects were to be expected. Nevertheless 1 patient suffered from abdominal distension and diarrhoea for 1 day, while another had several liquid stools for the whole 8 days of

treatment Somewhat later, Shookoff and Sterman (1952) drew attention to the few trials which had been made of bacitracin in conjunction with an amoebicidal agent In one such trial made by Graupner, Smith, and Priest (1950) a recurrence could not be prevented In Shookoff and Sterman's series of 22 cases treated with 60,000 Units a day by mouth for 5 days failures occurred in 5 patients but these were successfully treated with larger doses of the drug

Pinworm infestation

In spite of the unpromising results of the experiments described by Wells (1952 b) with *Aspicularis tetraptera* infections in mice, Chan and Brown (1953) attempted to rid patients of *Enterobius vermicularis* by a combined treatment of bacitracin and succinylsulphathiazole The diagnosis was confirmed by finding ova in the stools of each case Both drugs were given by mouth for 5 days to 17 patients and for 7 days to 11 patients Twenty three of the 28 patients showed rectal swabs free of ova during 3 weeks following treatment, but more of those patients treated for 7 days recovered than those treated for only 5 days Loose stools appeared in a few cases during treatment, but these did not necessitate discontinuance of drug therapy Again it is to be questioned whether the effect of these drugs was on the pinworm itself or an indirect effect due to their action on the bacteria in the faeces

Infections within the Chest

Bacterial endocarditis

There appears to have been only 1 case of bacterial endocarditis which was treated from the start with bacitracin This was a patient described by Applebaum, Bruno, and Hochstein (1952) This patient was treated initially in 1948 for pneumonia, but already had at this time a systolic murmur distinguishable at the apex of the heart A culture of *Str pneumoniae* type II was grown from the blood Treatment was begun with doses calculated not to cause any serious damage to the kidneys, namely 33,000 Units 6 hourly by intramuscular injection Although the patient seemed to improve for the first 3 days of treatment his fever rose Doses were none the less steadily increased until 98,000 Units were being given every 4 hours This produced a temporary improvement, but 3 days later systolic and diastolic murmurs were heard over the aortic area, and although penicillin, together with caronamide, were substituted for bacitracin and the blood culture at last became sterile, the patient died 9 days later from congestive heart failure At autopsy lesions were seen in the endocardium, the right and left coronary leaflets were almost completely destroyed by large ulcerative vegetations, and one of these had perforated In addition to these lesions, however, a toxic nephrosis was also present, due either to the acute infective process or to the high doses of bacitracin

Other accounts have given promising demonstrations of what bacitracin can do following or combined with penicillin treatment Friedberg and Bader (1951) successfully treated a staphylococcal infection, Volini and Kadison (1951) an infection due to an enterococcus and 2 due to streptococci, Wallach and Pomerantz (1951) and Loewe, Cohen, and Liber (1953) infections

due to *Str. viridans*, Zendel and Lubart (1952) a case from whom only diptheroids could be isolated, and Schwarz and Lazarus (1950) an infection due to an anaerobic Gram positive coccus. It is of interest to note that although synergism between bacitracin and penicillin against the responsible organism was demonstrated *in vitro* by both Schwarz and Lazarus (1950) and by Wallach and Pomerantz (1951) none of these workers attempted to use bacitracin until other antibiotic treatment had not produced a response. Nevertheless, whether bacitracin was given with penicillin, dihydrostreptomycin or aureomycin, or after these other antibiotics, not more than 80,000 Units in divided doses were administered to any of these 8 cases daily, and often as little as 30,000 Units combined with penicillin was sufficient to rid the blood stream of organisms, and to ensure the recovery of the patient. One other case, reported by Jenkins, Uhr, and Bryer (1954), needs further examination. This was a woman of 57 with some degree of congestive heart failure, all the other cases previously mentioned were under 50 years of age. The illness began 3 months previously, after the extraction of 6 teeth for pyorrhoea, and blood culture revealed a non haemolytic streptococcus on 1 occasion only. Intramuscular penicillin followed by 2 G of terramycin daily did not relieve the fever, and no demonstrable benefit was produced by 4.8 million Units of penicillin and 1 to 2 G daily of *Benemid*, together with streptomycin or dihydrostreptomycin. After 30 days of this latter treatment the patient was still in a state of congestive failure with fever, when bacitracin was administered in addition to the other drugs. The dose was limited to 50,000 Units daily given in 5 injections. The patient became afebrile in 3 days and remained so for the 6 days during which bacitracin was given. Within 3 days, however, oliguria had developed, the blood urea nitrogen rose from 44 to 76 mg per cent and the amount of albumin in the urine increased. Death occurred 10 days after the beginning of bacitracin treatment. *Post mortem*, the vegetations found on the aortic cusps were sterile and appeared to be healing but microscopic examination of the kidneys showed swelling, eosinophilia, and hyaline droplet formation of the epithelium of the proximal convoluted tubules, and in some tubules there was necrosis, desquamation, and cast formation. Jenkins *et al* (1954) concluded that the toxic action of bacitracin on the kidneys of this patient, who already had congestive heart failure, was responsible for her death.

From these few reports it appears that bacitracin, used in conjunction with penicillin, seems to be a remarkably effective agent against a susceptible infection, but high doses are inadvisable even in a patient with relatively normal kidneys, and the drug may well be contraindicated in patients suffering from congestive heart failure. In the latter cases, the antibiotic should only be used with extreme caution.

Pneumonia

Although a few cases have been quoted as having been treated successfully in mixed series of clinical trials, only 1 study appears to have been devoted to the use of bacitracin in this relatively common infection. This was carried out by Reisner *et al* (1951). These authors chose 14 cases with acute lobar pneumonia for study. Pneumococci were isolated in 10 of their patients and haemolytic streptococci in 3, while in 1 case no organisms could be cultivated. Bacitracin was administered in a relatively high dosage to begin with

(up to 99,000 Units intramuscularly every 6 hours), but as soon as some response was noted in the patients the dose was lowered to between 30 000 and 50,000 Units 6 hourly. Twelve of these patients recovered from their pneumonia, and the 1 case which failed to make a response was subsequently successfully treated with penicillin. In the other case, which was due to pneumococcus type II, endocarditis developed after the pneumonia had resolved, and the patient died in spite of penicillin being administered. The cause of death was considered to be heart failure. This case history is similar to one described in greater detail in the section on endocarditis. Two patients, although they eventually recovered, developed pleural effusions. In one of these the effusion, although sterile, was loculated and difficult of access, the drug was therefore given as an aerosol in doses of 10,000 Units 3 times daily. With this aerosol therapy the temperature fell promptly to below 100° F and cough and sputum steadily decreased. In these patients there were, however, some toxic manifestations. Eleven had considerable albuminuria which, however, ceased within 4 days of discontinuing treatment, except in the patient with endocarditis who continued to excrete albumin until death. The excretion of phenolsulphonphthalein was impaired in all these cases but returned to about normal during the 2 weeks following treatment. Besides these more serious side effects an urticarial skin reaction developed in the patient who received aerosol treatment, and some patients suffered from painful and swollen buttocks after several injections. In 1 patient a low fever appeared an hour after each injection. There are still no results in series treated with a lower dose where toxic effects on the kidneys are not so likely to play a part in hindering convalescence.

Pericarditis

The case of a child of 7 was described by Freeman and Parker (1953). Pericarditis developed without apparent cause and when an organism was finally isolated from the pericardial fluid it was found to be a *Staph albus*, coagulase negative. When aqueous procaine penicillin and chlortetracycline had been administered without alleviation of the symptoms, the organism was tested for sensitivity *in vitro* to various antibiotics. It was found to be resistant to all except bacitracin. This antibiotic was then administered in doses of 400 mg per kg or 6 000 Units 6 hourly by intramuscular injection, later 10 000 Units 6 hourly were given for 3 weeks. Although the fever did not subside immediately, the child improved gradually and paracentesis was not required. The temperature became normal 6 days after the higher dose of bacitracin had been given, the friction rub diminished, and the heart sounds became stronger and more regular. A month after treatment the radiograph showed a normal heart shadow without any residual signs of the atelectasis which had developed as a result of the enlargement of the heart and pericardial sac. The electrocardiograph was within normal limits. Nine months after discharge the child still had a systolic murmur over the mitral area but he was well and active.

Infections of the Central Nervous System

After injections of 10 000 Units of bacitracin were made into the cisterna magna of dogs without producing any obviously damaging effects. Teng

and Meleney (1949) turned their attention to the treatment of staphylococcal meningitis with bacitracin. These investigators had already demonstrated the ability of the antibiotic administered by the cisternal route to protect dogs from lethal staphylococcal infections (Teng and Meleney, 1950 *a*), and they were able to demonstrate the therapeutic effect of the drug in 5 cases of staphylococcal meningitis in man. These had all been treated unsuccessfully with other agents when bacitracin was employed. All recovered completely and confirmed the belief of these workers that bacitracin was much less toxic than other antibiotics when given by the intrathecal, intracranial, or intraventricular routes (Teng and Meleney, 1950 *b*). By 1953 some 70 patients with infections involving the central nervous system had been treated by Teng, Cohen, and Meleney (1951) and Teng and Meleney (1953). Nine of these were suffering from osteomyelitis of the skull with epidural abscess due to penicillin resistant staphylococci. When the infected tissues had been removed as far as possible by surgical means bacitracin was applied locally and also injected intramuscularly. All of these patients recovered.

Seventeen cases of meningitis due to various organisms were treated with the antibiotic in a similar way, sometimes with the addition of preteral penicillin. Thirteen of these cases survived, but 4 died from such causes as pineal tumour or mid cerebral arterial thrombosis.

Seven cases of brain abscess were treated and 6 of these survived.

Twelve laminectomy or craniotomy wounds infected with penicillin resistant staphylococci responded to treatment and healed completely after bacitracin was used.

Some of these patients were treated by local applications alone, and none of them showed urinary changes. Intrathecal or even intracerebral injections did not produce untoward effects when used prophylactically, for example in compound fractures of the skull or in contaminated craniotomy wounds. When the local applications were supplemented by intramuscular injections of 10,000 to 20,000 Units 6 to 8 hourly, transient albuminuria and cylindruria appeared in some of the cases.

Synergistic Gangrene

This entity was mentioned by Meleney in all of his clinical trials, and was described in detail by Meleney, Shambaugh, and Millen (1950). Five cases in various stages of the disease were described from early gangrene to loss of the whole skin of the lower abdomen, flanks, and upper thighs. The responsible organisms in 3 patients appeared to be staphylococci and non haemolytic micro aerophilic streptococci. In none of these patients were any of the tissues excised once bacitracin therapy was begun. The antibiotic was administered in doses of 10,000 to 24,500 Units 6 hourly by intramuscular injection and applied locally in concentrations of 500 to 1,000 Units per ml for periods varying from 11 to 30 days. As treatment proceeded the epithelium was seen to be growing out from residual islands on the denuded surface. In 1 case the extent of the gangrene was too great to obtain an epithelial covering by this method and skin grafts were applied (Fig 5, *a*, *b*, and *c*).



FIG 5a and b (see over)

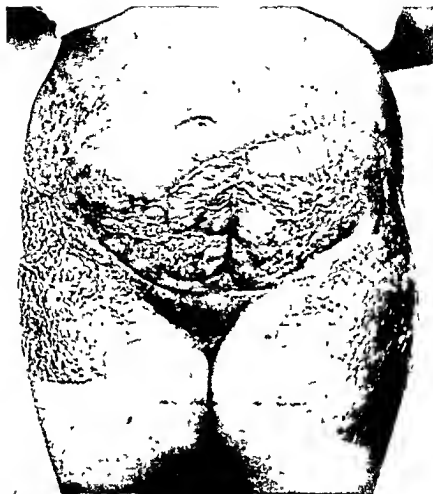


FIG 5c

- a Synergistic gangrene in a patient 2½ months from first appearance. An attempt to stop the progress of the infection has been made by cutting a trench round it and packing it with gauze soaked in penicillin.
- b 10½ months from onset. Extension of lesion down both thighs. Before treatment with bacitracin *Staph aureus* resistant to penicillin and sensitive to bacitracin, *S. proteus* and *Ps. pyocyanea* were isolated.
- c Appearance 7 weeks from starting bacitracin. complete healing has taken place.
(From Melney Shambhugh and Millen *Ann Surg* 1950 131, 129)

Wound Infections

Pulaski and Connell (1949) have had the most extensive experience in the treatment of wound infection. In 26 patients of military age, 23 with acute wound infections and 3 with chronic infections, these authors administered bacitracin by intramuscular injection every 6 hours in doses of 10,000 to 20,000 Units, together with a 2 per cent solution of procaine in saline. The infection subsided in 18 of these cases but in the remainder the effect of bacitracin was doubtful or unsatisfactory. These workers attributed the poor response in these latter cases to organisms which were resistant to bacitracin or else to the presence of avascular tissue around the lesions, which prevented access of the drug to the infected part. The toxic effects of the drug at that time, however, were enough to preclude its further use.

Gastro-intestinal Infections

Dysentery

Silverman (1951) treated 14 cases of dysentery due to shigellae. The Duval Sonne strain, the Flexner strain, or the Shiga strain was isolated from the faeces. Forty thousand Units of bacitracin were administered 3 times daily by mouth for 8 to 30 days. No toxic symptoms were noted and dysentery organisms could not be found in the stools at the end of treatment. There were no relapses during the following 3 months to 3 years. As dysentery bacilli are not susceptible to bacitracin *in vitro* it is possible that the drug exerted some indirect effect on these organisms through its action on other faecal flora.

Although the causative organism was not known, Kadison and Borovsky (1951) treated 53 cases of diarrhoea in infants of less than 1 year with a combination of bacitracin and neomycin. The drugs were given by mouth in doses of 10,000 Units of neomycin and 2,000 Units of bacitracin crushed in apple jelly, every 6 hours for 4 to 14 days. As usual, no absorption into the blood stream was detected and no renal damage or side effects were observed. Damage cleared up in an average of $3\frac{1}{2}$ days compared with 6 $\frac{1}{2}$ days in 32 patients treated with streptomycin and procaine penicillin. It is again difficult to attribute the cure of the diarrhoea to the elimination of any particular susceptible organism. In any case, as both *Ps. aeruginosa* and *Proteus morganii* persisted in the stools after treatment, it was assumed that neither of these was responsible for the diarrhoea.

Pre-operative prophylaxis

The problem of rendering the gut sterile before carrying out an operation on it has exercised the minds of many investigators. For this purpose a combination of neomycin and bacitracin was employed by Moritsch (1955). Acting on the assumption that it was stenosis of the colon which prevented sterilization, Moritsch prepared 11 patients for operation by giving an enema and an antiseptic bowel washout as well as drug therapy. One or 2 tablets each containing 0.25 G. of neomycin and 12,500 Units of bacitracin were given 3 times a day for 3 days. It is of interest to note that in the stools of patients receiving the smaller dose no bacteria were found or only *B. subtilis* or *E. coli* and staphylococci, while those of patients receiving the larger dose

contained no cocci, enterococci, *E. coli*, or clostridia. Moritsch (1955), however, pointed out that any obstruction to the passage of faeces through the gastro intestinal canal interfered with sterilization of the bowel contents. As an example he quoted 2 patients in another series who had stenosis in some part of their colon. From the faeces of these patients both *E. coli* and enterococci were isolated despite treatment.

Urinary Tract Infections

Because of its nephrotoxic effect bacitracin could not be considered as a suitable drug for urinary infections when given parenterally. Wechsler and Frishwasser (1953), however, treated a series of 16 cases with severe cystitis of the bladder by local instillations of saline containing 500 Units of bacitracin per ml. Although the majority of the organisms isolated from the urine in these cases was Gram negative, it is possible that the proteus bacilli may have overgrown the coexisting staphylococci or streptococci during culture. Whether or not this was the case, the urine became clear in all cases within 3 days, and cultures became sterile at the same time. Rapid healing of suprapubic wounds also followed local application of bacitracin.

Infections of the Eyes

Experimental work with instillations of bacitracin into the eyes of rabbits showed that the antibiotic produced little irritation (Bellows and Farmer, 1948 a). Even the dry powder when applied to the cornea of the animals for 30 minutes and then washed out caused no gross irritation. The drug was found to penetrate the cornea when it was abraded or inflamed but no penetration occurred through a normal membrane. Injection of 0.1 ml of saline containing 100 Units of bacitracin into the vitreous produced moderate degrees of opacity which, however, cleared away in 6 out of 8 eyes during the following month. Bellows and Farmer (1948 b) did not have the opportunity of testing all these findings by clinical trial, but they were able to test the effect of bacitracin on infections on the surface of the eye. These investigators established first that frequently repeated instillations of a solution containing 1 000 Units of bacitracin per ml in saline protected rabbits' eyes from infections with a susceptible strain of *Staph. aureus*. Forty three patients with external ocular infections were then treated. Bellows and Farmer (1948 b) found that acute conjunctivitis responded to treatment in 11 out of 16 cases, acute kerato conjunctivitis in 2 out of 3 cases, acute exacerbations of chronic conjunctivitis in all of 5 cases and chronic conjunctivitis in only 6 out of 18 cases. Curiously enough, a corneal ulcer in 1 patient in which *Ps. pyocyanea* was found recovered rapidly after instillations of bacitracin. These workers stated that recovery was rapid in acute conditions in which the infecting organism was found to be susceptible to bacitracin but response was less satisfactory when the organism was only slightly susceptible. A more enthusiastic report on the effect of local applications of bacitracin to the eye was made by Eggers (1951). This author employed the solution or the ointment in more than 400 patients and asserted that all patients with conjunctivitis, blepharitis, dacryocystitis and corneal ulceration benefited from the treatment and that the drug gave effective prophylaxis in cases involving the removal of foreign bodies from the cornea and in

most operative procedures. In 1 case only was there any sign of irritation or sensitization to the drug.

Ear, Nose, and Throat Infections

Topical application of bacitracin was tried by Coyle, Collins, and Nungester (1949) in 43 patients with ear, nose, and throat infections. Patients with mild pharyngeal infections appeared to benefit from this type of treatment, but poor results were obtained in external otitis or chronic suppurating mastoid cavities. Most of the latter were mixed infections so that correlation between bacteriological findings and clinical effect was difficult.

A combination of bacitracin with polymyxin was instilled into the nose in a series of patients suffering from the common cold by Clark (1954). Although it is possible to find some reason for the relief of symptoms said to have been experienced by patients who had suffered from the colds for several weeks, it is more difficult to understand why patients with acute infections were also said to have been relieved.

Infections of the Skin

Some of the earliest workers to investigate the usefulness of bacitracin in skin lesions were Miller *et al* (1948). These authors used an ointment base containing 500 or 1,000 Units per G in 165 patients. Sixty eight of these suffered from primary bacterial infections of the skin, and the others from infected dermatoses. Fifty of the former group responded favourably but failures were recorded in 18, 11 of whom had folliculitis of the beard. In these, however, the condition at least did not spread while treatment was continued. Failures also occurred in patients with eczematoid dermatitis, vesiculo pustular eruptions on the hands, and folliculitis in areas other than the beard. Higher concentrations of the drug appeared to be no more effective than lower concentrations. As is usual with antibiotics in infected dermatoses, the infection often cleared up but, there was no effect on the dermatosis. In the cases with folliculitis it is possible that failure of the local application to penetrate into the depths of each hair follicle was responsible for the lack of response. Derzavis, Rice, and Leland (1949) also tried local application of bacitracin in an ointment base. Except in cases of impetigo, ecthyma, and infectious eczematoid dermatitis the results were not outstandingly good. Only 1 patient among 138 showed any sign of sensitization to bacitracin. Other workers who tested the effect of bacitracin on infections of the skin were Eichenlaub and Olivo (1949), Finnerty (1951), and Wrong Smith Hudson, and Hair (1951). The conclusions of these authors were similar. Lesions which were accessible to the applications became free of infection in a materially shorter time than that obtained with previous treatments, but although the incidence of sensitization reactions was low, these still occurred. If bacitracin were more widely used it is questionable whether the incidence of sensitization might not increase.

Treatment with bacitracin combined with another antibiotic

Some mention of this has already been made. When it was realized that Gram negative bacteria could be isolated from a certain proportion of skin lesions (for example, Kile *et al* 1953, and Farrar, 1954), the use of bacitracin

combined with some agent active against these organisms was considered Rattner and Rodin (1952) compared the effect produced by a preparation of penicillin (L ephenamine benzylpenicillin) in an ointment base, with the effect of bacitracin combined with neomycin and another antibiotic, fradecin. Although these authors found that penicillin alone was most effective against primary infections secondary infections responded best to a combination of bacitracin and neomycin. This same combination in a preparation containing no more than 5 mg. of neomycin and 250 Units of bacitracin per G. of base (10 per cent lanolin in soft paraffin) was used by Gade *et al* (1953). Although much the most common organism isolated from the different lesions in this series was the staphylococcus, it was found in each instance to be sensitive to this combination of antibiotics. In 20 out of 74 patients treated with this preparation the response was not considered successful, but in 9 of these 20 a transitory response was none the less obtained. Montgomery and Montgomery (1953) did not consider that the average time taken to cure 50 cases of pyoderma treated with this mixture was less than that obtained with other antibiotics, but it was an improvement on the time taken by the older remedies. These authors also suggested that the occasional lesion infected with a strain resistant to other antibiotics might respond to this combination.

Polymyxin has also been combined with bacitracin in the local treatment of infections of the skin. The results obtained by Kile *et al* (1953) and by Pass and Rattner (1954) are described in the section on polymyxin. The report of Kile *et al* (1953) has the inestimable advantage of relating the clinical effect to the organism involved. Thus their figures showed how well the staphylococcus could in fact be controlled by this combination.

| Type of lesion | Total cases | No. of cases which improved according to infecting organism | | | | |
|----------------------------------|-------------|---|-----------|---------|---------|-------------|
| | | Staph | Str. faem | E. coli | Ps. pyo | Proteus sp. |
| Impetigo | 9 | 7 | | | | |
| Infectious eczematoid dermatitis | 3 | 1 | | 2 | | |
| Secondary pyogenic infection | 26 | 14 | 17* | 1 | 5 | 2 3* |
| Sycosis vulgaris | 4 | 3 | 1 | | | |
| Folliculitis | 5 | 3 | | | | |
| Miscellaneous | 5 | 2 | 3* | 1 | 1 | |

* Total of infections treated

From these figures it can be seen that the staphylococcus and proteus were the only organisms which could not be controlled in every instance by bacitracin and polymyxin. With this in mind, it should be preferable to change over to a combination of bacitracin and neomycin when lesions do not show an early response to treatment with bacitracin and polymyxin.

Ulcers

Chronic ulcers involving the whole thickness of the skin are a common problem. If it were possible to cure them by ambulatory treatment it would save a great deal of bed space in hospitals. Spier and Clifton (1954) were particularly satisfied with the results of 3 out of 6 patients with chronic ulcers treated by debridement by means of plasminogen, local applications of bacitracin and hyaluronidase. The necrotic bases of these ulcers were

cleared, and their surface thus rid of the pathogens most detrimental to healing. With the help of general measures calculated to reduce stasis, these ulcers had remained healed for 4 to 14 weeks at the time of reporting.

Tropical ulcers

This type of ulcer is most commonly infected with Vincent's organisms and these should respond to treatment with bacitracin. In a large series of African patients treated by O'Brien (1951) in Tanganyika, parenteral penicillin was administered together with local applications of various drugs. Bacitracin was one of the local applications used. In all these pyogenic ulcers, spread was arrested by means of antibiotic therapy and a diet containing adequate calories and vitamins, but O'Brien could not detect any marked difference in the healing rate between patients treated with penicillin and those treated with local applications of bacitracin. Nevertheless for patients who develop sensitization to penicillin, bacitracin may be a particularly valuable alternative.

Conclusion

From these few data it can be concluded that bacitracin is an excellent antibacterial agent for local administration, its effect in mixed infections being improved by the addition of neomycin or polymyxin. Given by mouth the drug has been of some use in amoebic colitis and dysentery due to various organisms, and it produces very few ill effects when administered in this way. There are risks attached to the use of bacitracin for parenteral administration, even in its present form. Nevertheless, as an adjuvant to penicillin in serious staphylococcal or streptococcal infections, for example in bacterial endocarditis, it can be of great value in controlling an otherwise resistant infection. In such cases 100,000 Units daily is the maximum amount to be administered.

POLYMYXIN

GENERAL CONSIDERATIONS

Three separate groups of workers isolated these antibiotics at about the same time. Ainsworth, Brown, and Brownlee (1947), Benedict and Langlykke (1947), and Stansly, Shepherd, and White (1947). The antibiotics were obtained from a soil bacillus called *B. aerosporus* by Ainsworth *et al.* (1947) and from *B. polymyxa* by Stansly *et al.* (1947). Comparison of the work in the laboratories of these different workers led to the conclusion that although the antibiotics isolated were derived from the same species of bacillus, chemically different polymyxins were produced according to the strain used. Thus 5 polymyxins have been described, called, for purposes of differentiation A, B, C, D, and E. All have similar antibacterial activity but differ in their toxic effects in experimental animals and man. All 5 have their most potent effect against Gram negative bacilli, but because of their toxic effects A, C, and D are not now used in man (Brownlee, Bushby, and Short, 1949). These antibiotics are basic polypeptides whose salts remain stable at a low

pH and withstand heating to 100° C for 1 hour (Jones, T S G, 1949, Pulaski, Baker, Rosenberg, and Connell, 1949, Regna, Solomons, Forscher, and Timreck, 1949, and Stansly and Brownlee, 1949) They are also soluble in water and methanol (Bell, Bone, English, Fellows, Howard, Rogers, Shepberd, and Winterbottom, 1949) In serum, polymyxin B and E lose 50 per cent of their activity at once It is possibly because of this effect that within 5 minutes of intravenous injection the blood levels of these polymyxins are only 10 per cent of those expected, assuming no loss into the tissue spaces or the body cells (Brownlee, Bushby, and Short, 1952)

The antibacterial activities of the several polymyxins have been tested by different workers The drugs are rapidly bactericidal *in vitro* (Bliss, Chandler, and Schoenbach, 1949 Jawetz, 1952, Brownlee, Bushby, and Short, 1952, and Brownlee and Bushby, 1948) The figures given in Table 11 are based on those given by Brownlee and Bushby (1948), but must be considered approximations only as different polymyxins were used by different workers It is curious to note that although so many Gram negative rods are sensitive to the action of the polymyxins, proteus has rarely been found to be sensitive The most highly susceptible organisms are the haemophilus and the salmonella groups, although it remains to be seen whether this finding *in vitro* is borne out in practice Weight for weight the effect of the polymyxins *in vitro* was almost invariably greater than that of streptomycin against susceptible organisms Another highly valuable property of these drugs is the fact that resistance develops only with difficulty when susceptible bacteria are exposed to increasing subinhibitory concentrations of the polymyxins (Pulaski *et al*, 1949, and Sberwood *et al*, 1953)

Animal protection experiments

These were carried out mainly with mice The results seemed to indicate that the polymyxins were valuable chemotherapeutic agents According to Brownlee and Bushby (1948) a majority of mice were protected from experimental infections with *Salm typhi*, *E coli*, *H pertussis*, *H influenzae*, and *Br bronchiseptica* by injections of 100 µg of polymyxin once or twice daily for 1 to 5 days Again Schoenbach, Bryer, Bliss and Long (1948) observed that mice infected with *Klebs pneumoniae* and *H influenzae* type B were protected by 1 mg per kg injected subcutaneously A curative effect was obtained in infections with *Neisseria intracellularis*, even though no action could be demonstrated against this organism *in vitro* Experiments were carried out by Larson, Carle, and Verder (1949) with *Br suis* infection and tularemia in mice When guinea pigs were infected intraperitoneally with *Br suis* and treated 19 to 20 days later with subcutaneous injections of polymyxin in doses of 6.4 mg per kg of body weight either once or twice a day for 9 or 10 days, no difference was noted between the spleens of controls and those of treated animals killed 1 day after discontinuance of treatment In a second series of experiments with *Br suis* infection, the animals were treated with polymyxin alone in doses of 1.5 mg 4 hourly for 10 days, or in combination with streptomycin or streptomycin and sulphadiazine However, there was no indication that the polymyxin had controlled the brucella infection

Experimental tularaemia Larson *et al* (1949) attempted to show that polymyxin had some curative effect on tularaemia In these experiments

mice were treated with the antibiotic within 24 hours of intraperitoneal inoculation with *Past tularensis*. All treated and control animals were dead within 120 hours of inoculation.

TABLE 11 ANTIBACTERIAL ACTIVITY OF POLYMYXIN OR AEROSPORIN ON STRAINS OF ORGANISMS PATHOGENIC TO MAN

| Organism | Inhibitory concentration (μg * per ml) |
|---|---|
| <i>Sensitive organisms</i> | |
| <i>Alcaligenes faecalis</i> | 0.4- > 4.0 |
| <i>Bact. coli</i> and <i>coli aerogenes</i> group | < 0.004-1.6 |
| <i>Bact. friedlanderi</i> or <i>Klebsiella pneumoniae</i> | 0.06-5.0 |
| <i>Br. bronchiseptica</i> | 0.125-0.15 |
| <i>Brucella</i> | 0.6-12.0 |
| <i>H. influenzae</i> | 0.006-0.6 |
| <i>H. pertussis</i> | 0.04-1.0 |
| <i>N. catarrhalis</i> | 0.08 |
| <i>Paracolobacter</i> | 1.2-1.6 |
| 'Paracolon' | 0.16 |
| <i>Past. pestis</i> and <i>multocida</i> | 0.4-1.0 |
| <i>Ps. pyocyanea</i> or <i>aeruginosa</i> | 0.1-3.1 |
| <i>Salm. typhi</i> | 0.08 |
| <i>Salm. paratyphi A</i> and <i>B</i> | < 0.04-0.08 |
| Other salmonellae | 0.06-0.64 |
| Shigellae | 0.08-0.6 |
| <i>V. cholerae</i> | 0.32 |
| <i>Insensitive organisms</i> (i.e. only inhibited by 10 to > 100 μg per ml) | |
| <i>Cl. welchii</i> | |
| <i>Corynebacteria</i> | |
| <i>Ery. rhusiopathiae</i> | |
| Fungi | |
| <i>Meningococcus</i> | |
| <i>Myc. tuberculosis</i> | |
| <i>N. gonorrhoeae</i> | |
| Pleuropneumonia like organisms | |
| <i>Proteus vulgaris</i> and <i>morganii</i> | |
| <i>Staph. aureus</i> | |
| <i>Str. pneumoniae</i> | |
| <i>Str. pyogenes</i> and other haemolytic streptococci | |

* 1 μg = 10 Units

From the data of Bradford and Day (1949), Bliss, Chandler, and Schoenbach (1949), Brownlee and Bushby (1948), Pulaski, Baker, Rosenberg, and Connell (1949), Levaditi, Vaisman, and Henry Eveno (1949 a), Florestano and Bahler (1952), Kagan, Krevsky, Milzer, and Locke (1951), McLean, Schwab, Hillegas, and Schlingman (1949), Sherwood, Delage, and Hermann (1953), White, Alverson, Baker, and Jackson (1949).

Experimental plague McLean *et al.* (1949) first observed that *Past. multocida* was susceptible to polymyxin *in vitro*. Gorzynski and Neter (1951) infected mice intraperitoneally with *Past. multocida* and gave 0.25 mg of polymyxin B subcutaneously within 12 hours of infection. All of the control animals were dead by the 4th day, whereas only 4 per cent of those treated with polymyxin B were dead at this time. By the 10th day however, 15 per cent had died.

Pseudom infection Wells (1952 a and b) studied the effect of various doses

of polymyxin B, chlortetracycline, and bacitracin on the number of *Aspicularis tetraptera* found in the stools of infected mice. Although it was thought that chlortetracycline and bacitracin had some effect, no effect was seen with polymyxin except possibly a reduction in the size of the worms.

Further experiments were carried out by Benhamou (1949) and by Levaditi, Vaisman, and Henry Eveno (1949) which confirmed the protective effect of polymyxin in mice infected with *Klebs pneumoniae*, *H influenzae*, *E coli*, *Ps pyocyanea*, *Salm typhi*, *Salm paratyphi A* and *B*.

Toxicity

It is in this field that clear distinctions can be made between the different polymyxins and their applicability to disease in man. It was generally agreed that polymyxins A, C, and D, because of their nephrotoxic effects, were not suitable for clinical use (see Stansly, 1949), but it was thought that polymyxins B and E were practicable from this point of view. The effects of these 2 polymyxins were studied in animals by Brownlee *et al* (1952). Further discussion will be confined to these 2 types unless otherwise stated. Polymyxin B is also known by the trade name *Aerosporin*.

Although a certain amount of proteinuria followed the administration of polymyxin B in rats, rabbits, dogs, and man, this effect was found to be reversible. With polymyxin E there was little evidence of kidney damage in most laboratory animals, but in dogs some hyaline droplet degeneration of the epithelium of the convoluted tubules together with some nuclear changes were still occasionally seen when the renal tissue was examined immediately after discontinuance of treatment. A few days later no such changes could be found. Other experimental tests in laboratory animals were carried out by Light, Tornabeni, and de Beer (1952) who confirmed the temporary nature of the effects produced by polymyxin B, as compared with the more permanent renal damage produced by polymyxin A in rats. Moyer, Mills, and Yow (1953), however, produced acute renal injury in laboratory animals when a sufficiently large dose of polymyxin B was injected. When intramuscular injections were limited to no more than 2.5 mg per kg per day, no significant changes in glomerular filtration rate, tubular reabsorption of glucose, renal blood flow, or excretion of potassium and sodium occurred. Twice this dose, however, showed some effects on both glomerular and tubular functions. Other organs did not seem to be affected.

In the meantime, Jawetz and Coleman (1949) had demonstrated that repeated intramuscular injections of polymyxin B in man could produce cumulative serum concentrations and that under these conditions toxic effects were seen in the central nervous system. As these authors were mainly treating patients with chronic infections of the urinary tract it is possible that the excretory function of their cases was often already impaired and that any toxic effects would therefore be accentuated. In 1951 Jawetz stated that a dose of 1 mg per kg of body weight given 12 hourly was most suitable, but that a nephrotoxic effect was produced if the dose was raised to 2 mg per kg for 7 days or even less. Transient paraesthesias, dizziness, ataxia, and weakness might then occur. Later still, Hopper, Jawetz, and Hinman (1953) described the effects of administering polymyxin B to 13 patients with long standing pyelonephritis. In those patients whose renal

function was essentially normal, in spite of a moderate proteinuria and some blood cells in the urine, a dose of 1.7 to 2.2 mg per kg of body weight per day could be given for 9 to 14 days by intramuscular injection, or even as much as 50 to 100 mg by intravenous infusion, without causing ill effects. When, however, there was any indication of depressed renal function, a lower dose of 0.8 to 1.3 mg per kg per day was sufficient to produce at least transient depression of endogenous creatinine clearance and some nitrogen retention.

Applying their experimental findings to clinical use, Yow and Moyer (1953) came to much the same conclusion regarding the daily intramuscular dose. Polymyxin B, was administered to 29 patients, who were suffering from infections due to *Ps. pyocyanea*, by intramuscular injections in doses amounting to not more than 2.5 mg per kg of body weight daily, with only transient albuminuria, pain at the injection site, paraesthesiae, or drug fever resulting. When renal tests were normal prior to drug therapy, they remained normal after treatment. Yow and Moyer (1953) therefore came to much the same conclusion as Javetz and his colleagues, namely that so long as kidney function was normal, intramuscular doses of polymyxin B might safely be given in doses up to 2.5 mg per kg of body weight per 24 hours.

From these specialized trials it is of interest to turn to the kinds of reaction observed in other types of patients. From this point of view the report of Pulaski *et al* (1949) is revealing. These authors employed only polymyxin B and E in the treatment of 50 patients with infections of the urinary tract, intestine, and wounds. When the dose did not exceed 2.5 mg per kg per day none of their patients developed true drug sensitization reactions and none showed evidence of any toxicity from either oral or local administration. However, as is usual after intramuscular injections, the patients complained of pain at the site of injection, paraesthesiae and hyperesthesiae mainly about the face and scalp. In some cases mild dizziness, weakness, and circumoral flush developed. These ill effects usually followed the 1st dose and persisted throughout treatment, but disappeared 24 hours after this was discontinued. In none of these patients were the symptoms severe enough to necessitate stopping treatment. With a daily dose of 2.5 mg per kg, Pulaski *et al* (1949) found no increase in blood-urea nitrogen or non protein nitrogen, nor was there any other evidence of renal damage. When, however, the dose was raised to 4 mg per kg or more there was albuminuria, red and white blood cells and sometimes renal cells in the urine. Even these toxic effects disappeared within 4 days of stopping treatment. These clinical investigations into the toxicity of polymyxin B and E had so far not made any distinction between the 2 drugs in this respect. It is therefore of interest to turn to the report of Swift and Bushby (1953) in which an attempt was made to differentiate between the effects of the 2 polymyxins. These authors confirmed the experimental data given by Brownlee *et al* (1952) showing that there was a demonstrable difference in the severity of the effects from the 2 polymyxins. Polymyxin B was administered to 32 patients and polymyxin E to 53 patients. The doses employed were 10,000 (1 mg) to 12,500 Units per kg of body-weight injected intramuscularly every 4 hours to children, and usually 250,000 Units to adults at the same intervals. As a result of this trial Swift and Bushby (1953)

considered that polymyxin B was not free from troublesome reactions such as pain at the sites of injection, pyrexia, malaise, and mild paraesthesia. There was no great difference in the effects produced by polymyxin E, if anything, pain on injection was less but the paraesthesiae were of the same extent. They concluded that these side effects were not sufficient to preclude the use of the antibiotics in clinical practice. They summarized the incidence of toxic reactions in their patients in the following manner:

| Type of polymyxin used | Total | Number of patients | | | |
|---------------------------|-------|----------------------|-------------------------|-----------------|----------------|
| | | With renal damage | With local reactions | With pyrexia | With rashes |
| A | 27 | 26 | 0 | 7 | 0 |
| B (W R L)* | 24 | 0 | 19 | 18 | 0 |
| B (Pfizer) | 8 | 0 | 6 | 3 | 6 |
| E | 53 | 0 | 8 | 4 | 2 |

* W R L Early lots supplied by the Wellcome Research Laboratories

Toxic effects from intrathecal injection

A description of a reaction following the intrathecal injection of 100,000 Units¹ of polymyxin B in a case of meningitis was given by McGill and Mendel (1953). The patient had developed a low grade meningitis following a frontal sinusitis due to *B. faecalis alkaligenes*, which was found to be sensitive to polymyxin B. Nine days after the first symptoms the patient received an intrathecal injection of polymyxin which was repeated twice a day. In addition 250,000 Units were given intramuscularly every 6 hours. Twelve hours after the first injection the meningism had increased and the cerebrospinal fluid, which before treatment had been clear, was now cloudy, but within 48 hours cultures of the cerebrospinal fluid were sterile. By the 8th day of treatment, the patient still had neck rigidity and urinary retention, but the cerebrospinal fluid was clearer and had fewer cells in it. Neck rigidity eventually disappeared but the patient developed generalized weakness of the legs and incontinence of bowels and bladder, which only slowly improved. Intrathecal treatment had been continued in spite of the symptoms for about 1 month. The authors considered that a generalized meningeal reaction resulted from the polymyxin and that this was followed by an arachnoiditis affecting the cauda equina. It was suggested that a lower dose might have been advisable, even though this dose of polymyxin B had been given intrathecally on previous occasions without provoking a reaction. The experimental work of Teng and Johnson (1953) supports the view that the polymyxin B was responsible for this reaction. These investigators found that the antimotic was both irritant and toxic when injected intrathecally in dogs, some of which died after injections of 40,000 Units. Smaller doses caused weakness of the hind legs and, when the animals were examined 1 month later, chromatolysis of some of the anterior horn cells with demyelination of the peripheral parts of the spinal cord were seen. Teng and Johnson (1953) describe 2 further cases. One was a newly born infant with meningitis due to *E. coli*, who had convulsions after an intrathecal injection of 5 mg (50,000 Units) of polymyxin B and the other was an adult man with otogenic meningitis, in whom 50,000 Units injected intrathecally

¹ 10 mg

produced backache and retention of urine for 5 days. Fortunately both recovered.

Administration

It had been shown that the polymyxins were poorly absorbed by animal tissues (Brownlee *et al*, 1952). Oral administration, except for action against organisms within the gut lumen thus seemed to be ruled out. When 2 mg per kg body weight was administered orally to 6 normal children no drug was found in the serum or urine during the following 4 hours (Kagan *et al*, 1951).

Intravenous administration

This had been used without any serious effect by Hopper *et al* (1953). Fifty to 100 mg of polymyxin B suspended in 250 ml of 5 per cent dextrose in water were administered, but the resulting blood levels were not reported.

Intramuscular administration

This is the method most used for systemic effects. Although relatively low blood levels are obtained in this way (Brownlee *et al*, 1952), it is the most practicable method of introducing the polymyxins into the blood stream. It is possibly because of the finding that 50 per cent of the activity of the drugs is lost immediately after they are mixed with serum *in vitro*, that the therapeutic results have not been as successful as might have been expected from their bactericidal action. Pulaski *et al* (1949) found that injections amounting to 2 to 4 mg per kg per 24 hours produced serum levels of about 1 to 8 μ g per ml. With 6 hourly injections a peak of 30 μ g per ml was reached after the 4th injection, but if the drug was given 12 hourly no cumulative effect was produced. Although only trivial amounts were excreted at first, the amount excreted increased 12 hours after administration began, and sometimes reached 40 to 400 μ g per ml. In children with pertussis, when a dose of 4.8 mg per kg per 24 hours was given in 4 hourly injections, Kaplan, Fischer, and Kohn (1949) found levels of 2.6 to 7.0 μ g per ml in the serum with no significant cumulative effect when administration was continued for 5 days.

Local administration

When applicable this had been by far the most effective method of using the polymyxins. Moreover the poor absorption of the drug by the tissues ensures that its action is only local and that it does not give rise to systemic toxic effects.

Aerosol administration

Kaplan *et al* (1949) used this method of administration to children with whooping cough. After repeated inhalations of 0.72 mg per kg of body weight at 6 hourly intervals, an average level of 2.3 μ g per ml was found in the blood, but no significant cumulative effect was observed over a period of 5 days. Kagan *et al* (1951) used this method of administration in 1 infant, 5.6 mg of the drug being dissolved in 1 ml of saline (0.8 mg per kg). Ten to 12 Units (1–1.2 μ g per ml) were detected in the serum during the following 2 hours.

Intrathecal administration

Again, because of the poor absorption of the polymyxins from other sites, this route is essential for the treatment of meningitis due to susceptible organisms. Injections of 5 mg (50,000 Units) of polymyxin B were capable of sterilizing the cerebrospinal fluid of an infant, but convulsions were produced. Injections of as much as 100,000 Units in a man have been followed eventually by recovery, but much lower doses than these have been given with good effect, for example, 20,000 Units (2 mg) have been injected repeatedly in an adult without producing signs of damage to the central nervous system.

CLINICAL TRIALS

Early clinical trials were carried out by Kagan (1949), Pulaski *et al* (1949), Ross, Burke, Rice, Bischoff, and Washington (1949), and Swift (1948). Since Gram negative rods invade the urinary tract so frequently, the polymyxins have been largely used for genito urinary infections. In only a few cases has treatment met with no response. Although in most cases *Ps. pyocyanea*, *E. coli*, *A. aerogenes*, and *Klebs. pneumoniae* disappeared from the urine within approximately 5 days and sometimes even within 12 hours of beginning treatment (Pulaski *et al*, 1949), the organisms usually returned at a later date (Hopper *et al* 1953). This return does not seem to have been due to any increase in the resistance of the organisms to the drug.

The results of treatment in gastro intestinal infections were disappointing, but since the drug was usually given by mouth in these conditions and absorption is poor, the uncertain results are understandable. *Salmonella typhi* were eliminated from the stools of carriers who were given 100 mg of the drug 4 hourly, but all these cases excreted typhoid bacilli again at variable periods following the cessation of treatment.

Meningitis due to Gram negative organisms could be treated successfully by intramuscular and intrathecal injections. Although the preliminary effects of intrathecal injections could be alarming, recovery from the infection eventually occurred. In pertussis the response was variable in spite of the fact that the organism was particularly susceptible *in vitro* to the antibiotic. Nevertheless, Swift (1948) claimed that infants responded dramatically when treated early enough.

Pertussis

Bradford and Day (1949) compared the relative effectiveness of polymyxin B, chlortetracycline, and streptomycin in protecting mice from pertussis infection. Whereas streptomycin and polymyxin were equally effective *in vitro* the protective use of streptomycin was 10 to 50 times greater than that of polymyxin and approximated to that of chlortetracycline. Whooping cough was the first infection to be treated by polymyxin. Swift (1948) treated 10 infants and children suffering from whooping cough, but from only 1 of these could *H. pertussis* be isolated. Swift (1948) concluded that if polymyxin treatment was started within 1 week of the onset of whooping and continued in doses of 0.2 to 0.4 mg per kg 4 hourly, the children experienced an uncomplicated recovery apart from the toxic effects. A later report of Swift's

experience with polymyxin B given by Brownlee (1949) confirmed the earlier conclusions. These unfortunately were not fully supported by other workers. For example, in 17 children treated by Laurent with 0.5 mg per kg of body weight given in 4 hourly doses for 5 days (Brownlee, 1949), only 1 responded dramatically and this was a 17 month old infant with vomiting and severe paroxysms. Nevertheless, in half of the children the usual course of whooping cough was shortened. Again, Coutts, quoted by Brownlee (1949), treated 10 patients with proved pertussis with the same dose. In 6 of the children in whom treatment was begun before the 4th week of illness, the disease was aborted. A large series of cases was treated by Kaplan *et al* (1949). In each patient the diagnosis was made by the history and clinical findings or by the isolation of *H. pertussis* from the nasopharyngeal swab (34 cases). To these children polymyxin B was administered in doses of 4.8 mg per kg of body weight per day given in 4 hourly doses by intramuscular injection, or 60 per cent of this dose by aerosol. These authors considered that 23 of their 66 patients were undoubtedly improved by intramuscular treatment, and 5 out of 18 by aerosol administration.

The most significant finding in these equivocal results was the number of complications. In 9 cases there were pulmonary complications: atelectasis, pneumonia, or emphysema. Four children had diarrhoea and 2 had otitis media. There is no evidence to suggest that these conditions were due to insusceptible infection, but experience with other antibiotics would lead one to suspect that these secondary conditions might well be due to such organisms.

In studying the primary disease, Kaplan *et al* (1949) stated that the paroxysms in 55 of their patients reached their peak in the 3rd week and then gradually diminished in number and severity until the 6th. The children gained weight slowly, and vomiting continued to accompany the paroxysms. These authors drew attention to the fact that the mortality from pertussis had declined during recent years even without treatment. All these reports lead one to the conclusion that the only time when one could expect polymyxin to exert a chemotherapeutic effect would be in the early stages, before the paroxysmal stage had been reached. Except in an epidemic, administration of the drug at this stage would seem to be a counsel of perfection which is hardly practicable at the moment.

Typhoid fever

Four patients with typhoid fever were treated with polymyxin by Knight, Ruiz Sanchez, Ruiz Sánchez Schultz, and McDermott (1950a). Unlike other patients treated by chlortetracycline and chloramphenicol, these 4 cases showed no benefit which could be attributed to the antibiotic. Treatment was continued in all for 45 days even though the poor absorption of polymyxin by mouth was unlikely to affect the infection.

Meningitis

The first case to be treated by polymyxin B was a child of 13 months with an infection due to *H. influenzae* B which was sensitive to 0.43 Units per ml of the antibiotic. The organism was not sensitive to less than 80 µg per ml of streptomycin (Kagan 1949). After the diagnosis had been made, large

doses of streptomycin, sulphadiazine, and anti-influenzal rabbit serum were given, but without success. After 2 weeks of this therapy, during which time the child's condition steadily deteriorated, treatment with polymyxin B was substituted. This was given by intramuscular injection every 4 hours in a dose of 7 mg dissolved in 1 ml of diluent and intrathecally in a dose of 1 mg in 0.5 ml saline on the 1st day, 3.5 mg on the following days. As a result of this treatment, the child's condition appeared to deteriorate for the first 4 days and then showed rapid improvement. Three months after recovery and discharge from hospital, no neurological or other physical defects could be found, although the mental development of the child appeared to be retarded. Other cases of meningitis due to *H. influenzae* were reported by Brakeley (1950) whose patient, aged 3½ years, recovered after 10 days' treatment with 100,000 Units of polymyxin 3 hourly by intramuscular injection and 4 days' treatment by intrathecal injections of 10,000 to 35,000 Units and those by Swift and Bushby (1951). The latter workers treated 8 children in whom *H. influenzae* B had been isolated from the cerebrospinal fluid. The sensitivity of the organisms *in vitro* was 1.25 to 6 Units per ml. On the 2nd to the 8th day after symptoms had appeared these children were given 7,500 to 12,500 Units of polymyxin E per kg (0.75 to 1.25 mg per kg) by intramuscular injection 4 hourly. In addition, intrathecal injections of 0.5 to 4 mg were administered daily according to the age of the patients, which varied from 5 months to 3 years. In all the cerebrospinal fluid was sterilized within 72 hours. Seven cases recovered completely while 1 died from an intercurrent pneumococcal infection. It is of interest to note that, with these intrathecal doses, no ill effects were observed except for a temporary hypotonia in 3 patients. The pneumococcal infection, however, was a serious complication which might have been foreseen from the fact that the child originally had an upper respiratory infection which had responded within 48 hours to penicillin and a sulphonamide, both of which were discontinued after polymyxin was administered. This complication with so specific a drug as polymyxin casts some doubt on whether one ought to rely on it alone to control an infective process. Fig. 6 shows the chart of 1 of these children to whom penicillin was given, as well as polymyxin, to counteract any accompanying coccal infection.

Meningitis due to Ps. pyocyanea

This condition rarely appears to arise unless there has been some penetration of the intrathecal space from outside. Hayes and Yow (1950) described a case in a young woman of 17 who developed fever, backache, headache, and pain between the shoulders 3 days after spinal anaesthesia had been given for an appendicectomy. Although the cerebrospinal fluid was examined on the 8th post-operative day it was not until 2 months later that *Ps. aeruginosa* was cultivated from it. No other antibiotic inhibited this organism in practicable concentrations, but it was very susceptible to polymyxin B. Accordingly, after 9 weeks of unavailing treatment with penicillin, dihydrostreptomycin, sulphadiazine, and chlortetracycline, polymyxin B was administered in doses of 80 mg by intramuscular injection every 6 hours and of 2 mg intrathecally every 12 hours for 3 injections. The cerebrospinal fluid became sterile within 24 hours of beginning treatment but again, 10 days later, cultures from it grew *Ps. aeruginosa*. This patient showed

considerable nausea, vomiting, and albuminuria during treatment. Generalized pruritus developed, and pains down the legs and in the back were troublesome. It was thought that some of these reactions were allergic in origin,

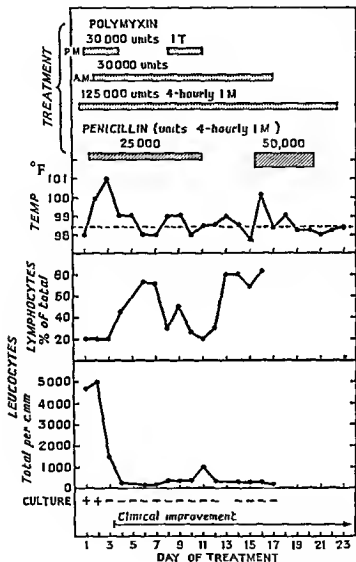


FIG 6 Meningitis due to *H. influenzae*, type B, in a boy of 18 months treated with polymyxin E and penicillin. From being semiconscious with neck rigidity, a positive Kernig's sign and a turbid cerebrospinal fluid from which the organism was isolated, he showed clinical improvement by the third day of treatment and his cerebrospinal fluid was free of *H. influenzae* in less time than this. Penicillin was given for a widespread bronchitis and to counteract the superinfection of any coccal infection.

(From Swift and Bushby *Lancet*, 1951, ii 183)

for the patient seemed to benefit from taking pyribenzamine. It was 6 months before normal health and weight were regained. Another patient reported by Tomlin (1951) contracted the same type of meningitis following an endoscopy for renal colic. The onset was again not particularly acute, for the patient had had a bitemporal headache for a month before the

cerebrospinal fluid was examined. Again other antibiotics were administered in a vain endeavour to control the meningism, until haemorrhages in the fundi, early papilloedema, and a headache confined to the right supraorbital area raised the suspicion of a frontal lobe abscess. *Ps. pyocyanea*, which had been cultured on occasion from the spinal fluid, was now cultured from the ventricular fluid. Intramuscular injections of polymyxin B, amounting to 3.2 mg per kg per day, were given 4 hourly, and 5 mg were injected intrathecally each day for 3 days. The patient suffered from severe pain in his legs after the intrathecal injections and also at the sites of intramuscular injection, and this was accompanied for 15 minutes by circumoral and perioral anaesthesia. A faint trace of albuminuria appeared during the 2nd day of treatment, but after 7 days of treatment the patient began to show signs of improvement. No *Ps. pyocyanea* had been cultured from the cerebrospinal fluid after the 1st day. In spite of the parenteral administration, the urine was not sterilized so readily and it was necessary to use other drugs before it became sterile. Two and a quarter months after treatment, this patient was discharged from hospital and had gained weight, but the haemorrhages in the fundi were still apparent for 6 months. Lastly, Trapnell (1954) described a case of *Ps. pyocyanea* meningitis in a child of 5½ years following an exploratory operation in the cervical cord region. Ten days after the operation meningitis developed and the child was treated by penicillin, streptomycin, oxytetracycline, and chloramphenicol without effect. Before polymyxin B was administered a pure growth of *Ps. pyocyanea* had been cultivated from the cerebrospinal fluid. Treatment with 200 000 Units (20 mg) intramuscularly every 4 hours and 40 000 (4 mg) intrathecally 12 hourly for 3 doses, and then once daily, was followed by subsidence of temperature and improvement in the state of the cerebrospinal fluid, which became sterile by the 4th day of treatment. In spite of continued treatment for 8 days *Ps. pyocyanea* reappeared in the cerebrospinal fluid 3 days after treatment was stopped. A second course of the antibiotic, this time lasting for 26 days, ended in full recovery. In this child untoward symptoms and signs followed the administration of polymyxin B. Appetite was lost early, and vomiting was frequent. Sacral oedema developed on the 3rd day with mild but transient oedema of the face and a trace of albuminuria. After each intrathecal injection the child at first cried out from the pain which ran down his legs. Eosinophilia and xanthochromia of the cerebrospinal fluid developed during the 2nd course of treatment together with mild albuminuria. However, the blood urea remained normal and a week after the end of treatment the patient had so far recovered that he was kicking a football about the ward. Six months later he showed no evidence of relapse.

Conclusion

These cases of meningitis although all but 1 of them recovered produced symptoms alarming enough to make the intrathecal administration of polymyxin B a last resort as Bonzanigo and Molo (1953) describe it. Even though the patients of Swift and Bushby (1951) seem to have shown the mildest reactions from polymyxin E, the fact that hypotonia was produced in 3 of their 8 cases is an indication that, with less carefully controlled treatment, even polymyxin E may produce unfortunate results. No one else appears to have given the high doses administered by McGill and Mendel

(1953) by intrathecal injection. However, the fact that in their patient prolonged convalescence was required before motor and sensory faculties were again normal, and the records of other cases presented here, indicate that no dose of over 2 mg should be given intrathecally even though treatment needs to be repeated or continued for some time.

Infections of the genito-urinary tract

Pulaski and Rosenberg (1949) found that polymyxin E given intramuscularly in doses of 2 to 4 mg per kg of body weight per day produced serum levels in the region of 8 μ g per ml from 30 minutes to 2 hours after injection, detectable concentrations still being present for 12 hours after a single dose. The concentration of the antibiotic in the urine did not reach its maximum for 12 hours, but after that time, with a dose of 3 mg per kg, a patient would usually excrete 40 to 400 μ g per ml of urine. With such concentrations in the urine many of the Gram negative species of bacteria should have been controlled by the drug.

Pulaski and Rosenberg (1949) were amongst the earliest workers to make a trial of polymyxin B in urinary tract infections. They chose 20 patients with severe and intractable infections and gave them 2 to 5.6 mg per kg per day for 2 to 6 doses. The most satisfactory dose was considered to be 2.5 mg per kg given in 4 doses for 3 days by intramuscular injection with 1 per cent procaine. In 10 of these patients the bacteria disappeared from the urine, the fever subsided and symptoms and the gross and microscopic appearance of the urine improved. Moreover, during the following 3 weeks there was no relapse in these cases. Pulaski and Rosenberg (1949) considered that they obtained the best response in pyelonephritis due to *Ps. pyocyanea*, these organisms disappearing within 12 hours of beginning treatment. They had no success, however, in patients whose urine contained organisms resistant to polymyxin or in those with multiple abscesses of the kidney.

A further 8 cases were reported by Brownlee (1949). These included cases of cystitis, orchitis, prostatitis, and pyonephrosis, the infecting organisms being *Ps. aeruginosa* and/or *E. coli*, and various other organisms. All cases received 20 mg 4 hourly, which was enough to remove *Ps. aeruginosa* and other sensitive organisms from the urine within a matter of hours, but even with infections due to such a susceptible organism, 1 patient relapsed within 3 days of treatment. Nesbit and Baum (1950), in a comparative study of the effects of various antibacterial agents, considered that polymyxin should be reserved for infections due to *Ps. pyocyanea*, but suggested a combination of several antibiotics to deal with mixed infections.

Gastro-intestinal infections

Enteritis

When the responsible organism is known to be susceptible to polymyxin there seems a very good chance that acute infections will respond to this drug. Pulaski *et al* (1949) found that 200 to 400 mg per kg per day administered by mouth was sufficient to suppress all coliform organisms except proteus within 24 to 72 hours. The number of cocci present varied, while clostridia and monilia were unaffected. Not only were the coliforms removed,

but they did not return to the stools in recognizable numbers for 1 to 6 days after treatment ceased. Thus, if treatment were continued for 10 to 18 days, the stools could be kept free of these organisms for 11 to 24 days. Moreover, these workers found the coliforms susceptible to polymyxin both before treatment and also when they returned to the stools after the drug had been discontinued. A further trial in children was carried out by Kagan *et al* (1951). These authors, who treated their patients with intramuscular injections as well as by mouth, also found that polymyxin B could rid the stools of *Ps. pyocyanea* but the patients sometimes died from concurrent disease, or from the original enteritis when the drug had been discontinued early because of local and general toxic effects. Toxic effects mainly took the form of flushing, urticaria, and pain at the site of injection.

Dysentery due to shigella

Burke, Ross, Rice, and Washington (1949) treated 16 children suffering from this infection with a dose of 2 to 3 mg per kg of body-weight given 4 hourly for 7 to 15 days, either intramuscularly or by mouth. Fourteen of the children recovered quickly but in 2 the treatment produced no significant effect. Kagan *et al* (1951) treated 5 children with 2 mg per kg given by mouth every 4 hours. On this dose 2 children with Sonne dysentery showed no shigellae in the stools after 3 days' treatment and clinical improvement was manifest within 5 days. Three other children whose stools contained *Sh. alkalescens* were treated both by intramuscular injection and by mouth. In these cases the stools were free from shigellae after the 4th day and remained so, the treatment being continued for 7 days even though the children appeared clinically well after the 3rd day. A third series of 23 children under 11 years was collected by Lieberman and Jawetz (1951) from an institution for mental defectives where bacillary dysentery was endemic. All of these children were carrying *Sh. paradysenteriae*. The children were first isolated and then treated with polymyxin B or E by mouth 15 to 20 mg per kg daily, a similar dose to that given by Ross *et al* (1949) and Kagan *et al* (1951). The drug was given in 3 doses daily and continued for 10 days. Twenty of these children recovered and remained free of shigellae for the 8 weeks following the discontinuance of treatment. There were, however, 3 failures in whom the organisms disappeared at first but then returned to the stools within 3 to 6 weeks. No toxic effects were seen in this series so that repeated courses for carriers were considered a feasible procedure when necessary. Lieberman and Jawetz (1951) pointed out that as these polymyxins are not absorbed from the gastrointestinal canal in significant amounts and are not locally irritating, they form particularly useful agents for treating this recurring infection by mouth since they are highly bactericidal and stable.

Dysentery due to salmonella

With the same dose as that given to children suffering from shigella dysentery, Ross *et al* (1949) found only equivocal results in salmonella infections. One infant in the series described by Kagan *et al* (1951) suffering from a salmonella dysentery, received 5 days of intramuscular and oral treatment without any effect. Nevertheless in an adult patient reported by Brownlee

(1949), *Salm typhimurium* was successfully eliminated from the stools by doses of 100 mg of polymyxin B administered 4 hourly for 3 to 5 days, and clinical recovery took place. Similarly successful, although temporary, results were claimed for 4 typhoid carriers by Kay and McDonald (quoted by Brownlee, 1949). *Salm typhi* disappeared quite rapidly from the stools after 100 mg had been administered 4 hourly, but in all 4 cases the organism reappeared after variable periods of time.

When the origin of the enteritis was unknown and treatment was given on the chance that it might have some effect, results were not so satisfactory. Cathie (quoted by Brownlee, 1949) treated an unselected series of 20 infants in hospital amongst a number of others suffering from gastro enteritis. A dose of 50 mg was given by mouth daily for 4 days at 6 hourly intervals but no effect on the flora of the stools was observed. Nine of these infants recovered remarkably quickly, but the course in the remainder was in no way different from the usual progress of the disease when not treated by chemotherapy. Similar conclusions were reached by Burke *et al* (1949). Although these investigators noted a marked diminution in the stool flora on a dose of 2 to 3 mg per kg of body weight given 4 hourly for 7 to 15 days, no particular clinical benefit was observed in cases suffering from this type of diarrhoea. There is, however, an occasion when polymyxin may be of use for cases of non specific enteritis and that is when a specific bacterial infection supervenes on the original condition. Walker (1952) picked out 4 infants of less than 18 months in whose stools no distinctive bacteria had been isolated until serial examination in the 2nd or 3rd week after admission revealed *Ps aeruginosa* or a proteus sensitive to polymyxin. In addition to the supportive treatment which these patients were already having, polymyxin B was administered in doses of 4 mg 4 hourly for 5 days by intramuscular injection to the oldest infant and in doses of 50 mg 4 hourly for 10 days by mouth to the younger ones. In the first child treated by intramuscular injection, diarrhoea subsided within 48 hours but treatment was nevertheless continued for 5 days. Treatment by mouth was followed by a slower recovery, the diarrhoea subsiding in 6 days. These cases were not free from relapse. 1 case had a haematoma infected by the same organism for which treatment had been continued in all for 45 days, although polymyxin by mouth was unlikely to have an effect on the infected haematoma.

Preparation for bowel surgery

Acting on the demonstration by Jawetz, Coleman, and Gunnison (1954) that polymyxin and dihydrostreptomycin act synergistically, Elias, Ottey, and Moran (1957) administered each drug separately or the 2 together to 13 healthy adults, observing the effect on their faecal flora. Each group received medication for 4 days. Assays of faecal samples after doses of 32 mg daily of polymyxin and 800 mg of dihydrostreptomycin showed the presence of each antibiotic. Dihydrostreptomycin depressed the faecal count but with the 2 antibiotics it was depressed still further.

Burns

The role of polymyxin in infected burns seems to be largely that of controlling *Ps pyocyanea* infection. Jackson, Lowbury, and Topley (1951 b)

described how all their efforts with streptomycin, Phenoxetol, soluthiazole, and *p* bromophenylguanidine hydrochloride had failed to control the invasion of burns by this organism. Chlorotetracycline, chloramphenicol, and oxytetracycline had proved equally unsuccessful. At the end of 1948 these authors began to apply polymyxin E to burns every 2nd day in a 0.1 per cent cream or solution. With this preparation the results were encouraging compared with controls but not altogether satisfactory, for although *Ps. pyocyanea* disappeared from some burns by the 2nd day of treatment, it persisted in other cases and only 11 out of 21 cases were free from these organisms by the 5th to 7th day of treatment. A more concentrated preparation of the antibiotic was next used, a 1 per cent cream. This appeared to be more successful, but the presence of the antibiotic on the swabs taken from the burns may have interfered with the subsequent cultures. The 0.1 per cent preparations were then used as prophylactic applications and these were effective in keeping 158 of 162 burned surfaces free of *Ps. pyocyanea*, while more than 3 times this proportion of the controls had become infected with this organism.

When studying the effect of polymyxin E on the bacterial flora, Jackson *et al.* (1951 *b*) also attempted to relate the presence or absence of these organisms to the clinical progress of the lesion. These authors came to the conclusion that the absence of *Ps. pyocyanea* and other coliform organisms was associated with a significant reduction in the healing time of burns involving full thickness skin loss, the average healing time for those treated with polymyxin being 5.2 weeks and for controls 8.5 weeks. Moreover, grafts took more completely when the burned surfaces were prepared with polymyxin E. Furthermore the likelihood of death from *Ps. pyocyanea* or coliform septicaemia after the 7th day following the burning, was also diminished in patients treated with polymyxin E. These workers saw no unequivocal evidence that sensitization was induced either by local or parenteral polymyxin E nor was there any sign of toxicity except in 1 child with extensive burns who had a trace of albuminuria, which is, in any case, a common concomitant of extensive burning. By 1954 the effect of local applications of polymyxin had so convinced these workers of the value of the prevention of infection with *Ps. pyocyanea* that they excised and grafted all suitable burns immediately after admission. By this means they shortened by over 10 days the average healing time of the 50 to 100 cases received annually at the Birmingham Accident Hospital Burns Unit, compared with cases in which grafting was delayed for 3 weeks. The method used for differentiating between non viable and viable tissue at this early stage is described in their communication (Bull, Jackson, Lowhury, and Topley, 1954). Altuna (1955) described the serious effects that *Ps. pyocyanea* could produce in children when uncontrolled. In 680 burned children, 35 died from pyaemia due to this organism. Although the parenteral administration of polymyxin failed to cure these children, prophylactic local applications had already been shown to be of value by Jackson *et al.* (1951 *b*) and might have done much to prevent some of these cases from the serious after effects of their burns. Altuna (1955) tried a combination of tetracycline and polymyxin in relatively large doses in 4 of these children and 3 of these recovered.

Infections of the skin and ears

As Jawetz (1952) had pointed out, polymyxin B is most effective in eradicating susceptible infections when applied locally. Polymyxin was used alone in a series of cases of otitis externa by Farrar (1954). The trials were confined to cases infected with *Ps pyocyanea*, *Proteus vulgaris*, and *E coli*. Ten per cent of polymyxin B sulphate in propylene glycol acidified with acetic acid was supplied in a bottle with a dropper. The patients, who applied their own treatment at home, filled the affected ear with the solution while lying on the contralateral side and, after staying so for a short time, plugged the ear with cotton wool. The process was repeated morning and evening. In patients with unilateral otitis externa, one side was filled with solvent only. Farrar's results confirmed the effectiveness of polymyxin when applied locally to infections caused by *Ps pyocyanea*, but he did not find the drug so effective against *E coli* infections, and it was quite ineffective against *Proteus vulgaris*. Farrar's clinical results may be summarized as follows:

| Infecting organism | Total | No. of cases | |
|-------------------------|-------|---------------|--------|
| | | Dry in 7 days | Failed |
| <i>Proteus vulgaris</i> | 8 | 1 | 7 |
| <i>E coli</i> | 7 | 5 | 2 |
| <i>Ps pyocyanea</i> | 11 | 10 | 1 |

Of the 8 controls, none was free of the original organism at the end of the 7 day period. It is of interest to note that no sensitization reaction occurred in any of the cases. From these figures polymyxin would seem to have a particularly selective action and for this reason some investigators have adopted a combination of this antibiotic with another which was active against Gram positive organisms. This combination should be of particular benefit in infective dermatitis. For otitis externa Graves (1952) used a cream containing 8 000 Units (0.8 mg.) of polymyxin and 400 Units of bacitracin per G and found this highly effective in 100 consecutive cases. Kile, Rockwell, and Schwarz (1953) also used this combination of antibiotics in much the same concentration, 10,000 Units of polymyxin B with 500 Units of bacitracin in a petroleum base. The lesions in 78 of the 429 cases treated by these authors were examined bacteriologically. *Staph aureus* was the most common infective organism being present in 46 per cent, while *Ps pyocyanea* and other Gram negative bacteria were found in 13 per cent, of lesions. Twelve out of 13 strains of *Ps pyocyanea* found were inhibited by 1.5 to 6.2 Units per ml. of polymyxin, but when the 2 drugs were used together inhibition occurred at a lower concentration of polymyxin. It was therefore considered justifiable to incorporate polymyxin in the routine dressing. When summing up their results, Kile *et al.* (1953) stated that over 80 per cent of patients had benefited markedly or moderately from this treatment. An important point was that a few cases of irritation were seen. From the skin tests bacitracin seemed to be mainly responsible, no case of proven sensitization to polymyxin B was found. A third investigation with ointment containing the same constituents was carried out by Pass and Rattner (1954). These authors treated 577 consecutive cases of pyoderma with frequent applications of the ointment. The most responsive lesion, as remarked by others, was impetigo. Ecthyma, pyogenic paronychia, sycosis

vulgaris, and infected dermatoses responded more slowly, and only when the ointment was applied frequently enough. The underlying dermatosis was of course unaffected. In all these cases Pass and Rattner (1954) saw no reactions, 3 patients only complained of burning after the ointment was applied.

A combination of polymyxin and oxytetracycline was tried on 200 patients by Appel (1953). The preparation was an ointment containing 3 per cent oxytetracycline and 0.1 per cent polymyxin B sulphate in a petrolatum base. When applied 3 times a day after crusts had been removed by a detergent, the ointment cleared up all of 21 cases of impetigo in 2 to 10 days. Folliculitis also responded well, 15 of 18 cases being completely cleared. In those lesions where a bacterial infection had supervened on the original dermatosis, the superadded infection responded quickly but no effect was noted on the underlying dermatosis. In Appel's case of otitis externa, the condition cleared up in a few days although in some cases it had persisted previously for up to 5 months. Acne necrotica miliaris of the scalp also responded to this treatment, although more slowly. As might have been expected, pyogenic granuloma, chronic pustular acrodermatitis, exudative discoid dermatitis, sickle cell ulcer, palmar psoriasis, erythema multiforme, dermatophytosis and neurodermatitis circumscripta were unaffected by the treatment.

Another group of investigators who used a combination of oxytetracycline with polymyxin were Kullinger, Wynn, and Young (1954). Instead of an ointment these authors used a solution containing 25 mg of oxytetracycline and 50 000 Units (5 mg) of polymyxin B sulphate in 5 ml of propylene glycol. This was applied to the external ear of a patient infected with *Salm. florida*. The result was remarkable. After 1 day's treatment the patient was free from discomfort and in 2 weeks' time the ear appeared quite normal. Ausband (1955) also used a similar preparation for applications to both the external and middle ear. Of 23 cases with infected ears treated by instillation of 3 drops 4 times a day, 15 were cured, 2 improved and no change was observed in 6. Ausband observed that this combination seemed particularly effective against staphylococcal infections. Without further bacteriological data it is not possible to hazard a guess about the cause of the failure in the 6 patients who were not affected by treatment. An ointment, consisting of 30 mg of oxytetracycline and 10 000 Units (1 mg) of polymyxin B sulphate per G of ointment base, was used by Carsley (1955) on 75 patients with various types of pyoderma. The response in these cases was most rapid in cases with acute impetigo, folliculitis and sycosis barbae, and in all instances where superficial bacterial infection was present. Conditions which did not improve with this treatment were recurrent pyoderma of the hands, bullous impetigo, abscess of the face, acute pyoderma of the nose, chronic paronychia, pruritus ani, and nummular eczema. When the deeper layers of the skin were involved it was found necessary to administer oxytetracycline by mouth.

A combination of erythromycin with polymyxin E was applied by Laing and Scott (1954) as an ointment to 14 cases of pyoderma in children. The organisms isolated from these lesions were streptococci or staphylococci, all inhibited by 2.5 μ g per ml of erythromycin or less. The use of polymyxin would therefore seem to have been a prophylactic measure against Gram

negative infection, since the staphylococci and streptococci were eliminated by the erythromycin. The ointment was applied 3 times a day after removal of crusts. These workers reported that complete healing occurred within an average of 4-7 days; no sensitization was observed in any of their cases.

Infections of the eyes

In experimental work done in rabbits, Ainslie and Smith (1952) showed that polymyxin could be injected subconjunctivally. This procedure raised a bleb for 2 to 3 days, but did not affect the clarity of the cornea when the amount injected did not exceed 250 000 Units. Penetration into the aqueous humour occurred within 1 to 3 hours. Experimental infections and ulcers of the cornea of rabbits produced by *Ps. pyocyanea* were treated by daily subconjunctival injections of 500 000 Units for 4 days. This treatment was followed by healing but nebulae resulted. With half this dose protection could still be afforded against *pyocyanea* infection but not without production of a temporary corneal haze. Later, Ainslie (1953) treated 8 cases of keratitis in man. The organisms isolated in these cases were *Ps. pyocyanea* some times associated with a staphylococcus. Polymyxin E was only used after the eyes had failed to clear following treatment with penicillin, streptomycin, chloramphenicol, or oxytetracycline. Polymyxin was then given by subconjunctival injection in amounts of 200 000 to 250 000 Units daily or every 2 days. When hypopyon was present this was first evacuated. The ulcers healed, but sometimes recurred when treatment was limited to 2 days, but even after recurrence these ulcers responded to further treatment. In only 1 case had the eye to be eviscerated because of the extent of the lesion before polymyxin was applied.

These results were good when one considers the fact that ulceration must have been present for some time while various other antibiotics were being tried. It is of interest to note that an ointment containing chlortetracycline or oxytetracycline was applied simultaneously with the polymyxin injections. These antibiotics probably kept the staphylococcal infection in abeyance while the *Ps. pyocyanea* was being dealt with by the polymyxin.

Conclusion

From the foregoing results it would seem advisable to limit the therapeutic use of polymyxin to local application. Since oral administration is accompanied only by local effects this also is a legitimate method of employing the drug. Dysentery, particularly that due to shigella, should respond to doses of the order of 12 to 20 mg per kg of body weight daily divided into 4 hourly amounts. When an intractable infection, such as that of the urinary tract or meningitis, has to be dealt with for which no other means of cure can be found, it is justifiable to use polymyxin intramuscularly. Doses of 2.5 mg per kg per 24 hours administered in divided doses at 4 hourly intervals, can be employed. Intrathecal administration is only justified when preliminary tests have revealed the responsible organism to be susceptible only to polymyxin and should not rise above 2 mg with correspondingly lower doses for children and infants. It is better to discontinue intrathecal injections after 3 days and to recommence them if a relapse occurs than to continue daily injections for a longer time.

NEOMYCIN

GENERAL CONSIDERATIONS

Neomycin was announced by Waksman and Lechevalier in 1949 and was expected to replace streptomycin since it was much less liable to induce resistance in pathogenic organisms. It was isolated from a streptomyces, designated *S. fradiae* in Bergey's *Manual*, and was tested for various properties while still a somewhat impure product. Waksman and Lechevalier (1949) described it as a basic compound most active at an alkaline pH, soluble in water but not in organic solvents, and heat stable. The range of its antibacterial activity was wide but it did not exactly correspond to that of streptomycin. Moreover, it was equally active against streptomycin sensitive and streptomycin resistant bacteria (Waksman, Frankel, and Graessle, 1949 a). It had no action against fungi (Waksman, Lechevalier, and Harris, 1949 c). Table 12 gives an idea of the types of organism inhibited by neomycin. These include organisms found to be resistant to antibiotics other than streptomycin (Duncan, Clancy, and Hudson, 1950).

In animal protection tests Waksman and Lechevalier (1949) showed that mice or chick embryos could be protected from lethal doses of *Staph aureus* and salmonella infections. *Salmonella typhosa* infections could also be prevented by half the dose required with streptomycin. Tuberculosis in guinea pigs, when treated for up to 30 days, showed signs of repair and healing when the animals were killed 77 days after infection (Karlson, Cainer, and Feldman, 1950 b). Experiments with pinworm infection in mice by Wells (1952 a) demonstrated that neomycin given either before or after infection merely increased the 'worm burden' of the mice compared with that of controls. Neomycin was also effective against *Klebs pneumoniae*, *Proteus vulgaris*, *Ps. aeruginosa* (Hobby *et al.*, 1949), cholera in mice, rickettsial pox in guinea pigs to some extent, vaccinia in mice, and *E. histolytica* in young animals (Felsenfeld *et al.*, 1950 b). No protection, however, was afforded against *Trypanosoma cruzi* and *L. donovani* infections in guinea pigs nor *T. brucei* infections in mice.

The antibiotic was found to be bactericidal as well as bacteriostatic (Waksman *et al.*, 1949 c, and Waksman, Katz, and Lechevalier, 1950). It was able to kill organisms in the resting as well as in the logarithmic phase (Hobby *et al.*, 1949 a). Moreover, above the minimal bactericidal level the drug killed off the bacteria irrespective of its concentration or the number of organisms present, the only difference produced by greater concentrations was in the rate at which the bacterial population was destroyed (Schoenhard and Stafseth 1953). Substances which affected its activity in solution were sodium chloride, disodium phosphate, peptone, and dextrose (Schoenhard and Stafseth, 1953, and Gunnison, Kumshige and Jawetz, 1955). The latter workers also found that at 4° C the bactericidal effect was maintained as well as at 37° C for most bacteria but not for *Str. faecalis*, staphylococci, klebsiella, and *E. coli*.

Resistance

Although Waksman and Lechevalier (1949) claimed that resistance to neomycin could only be induced to a limited extent, other investigators

TABLE 12 ANTIBACTERIAL ACTIVITY OF NEOMYCIN *IN VITRO*

| | Units or μg per ml inhibiting organism | | |
|---------------------------------------|---|----------|-------------|
| | * | † | ‡ |
| <i>Aerobacter aerogenes</i> | 0.625-1.56 | | 0.62-25 |
| <i>B. anthracis</i> | 0.156 | | |
| <i>B. subtilis</i> | | < 1.20 | 0.078-0.312 |
| <i>Borrelia recurrentis</i> | | | 0.75 |
| <i>Brucellae</i> | 0.312-5.0 | | 0.62-50 |
| <i>Corynebacteria</i> | 0.156 | < 1.8 | 0.312-> 100 |
| <i>Cl. perfringens</i> | | > 30 | > 100 |
| <i>E. coli</i> | 1.3-5.0 | 1-> 30 | 0.31-20 |
| Enterococci | | 8 > 30 | |
| <i>E. histolytica</i> | | | 12.5-50 |
| <i>H. influenzae B</i> | 1.25-2.5 | | 2.5 |
| <i>H. pertussis</i> | 0.312-0.635 | | |
| <i>Klebs. pneumoniae</i> | 0.312-0.635 | < 1.8 | 0.31-25 |
| <i>Leptospira icterohaemorrhagiae</i> | | | 0.03-1.25 |
| <i>Listeria</i> | | | 0.75 |
| <i>Mycobacterium</i> | | | |
| H37 Rv | 0.1-1.0 | | |
| 607 (SM R) | 0.25 | | 0.03-2.5 |
| 7 freshly isolated strains | 0.44-0.68 | | |
| <i>N. intracellularis</i> | 1.25-2.5 | | |
| <i>Past. pestis</i> | 0.625 | | 0.7-1.25 |
| <i>Past. tularensis</i> | 0.156 | | |
| <i>Proteus</i> | 1.25-2.5 | 1 > 30 | 0.62-50 |
| <i>Ps. aeruginosa</i> | 3.54-25.0 | 1 > 30 | 0.62-50 |
| <i>Salmon. paratyphosa</i> | 0.64 | | 0.31-20 |
| <i>Salmon. typhosa</i> | 0.1-0.74 | | 0.31-5 |
| Other salmonellae | 0.4-0.625 | | 0.62-25 |
| <i>Shigellae</i> | 0.25-1.68 | | 0.31-20 |
| <i>Staph. aureus</i> | 0.156-0.74 | 1-20 | 0.3-100 |
| <i>Str. haemolyticus</i> | 1.50 | < 1-> 30 | 2.0-50 |
| <i>Str. dysenteriae</i> | 1.68 | | |
| <i>Str. pneumoniae</i> | 2.0 | 1 > 30 | 15.6 |
| <i>Str. viridans</i> | | 1-> 30 | 0.3 > 50 |
| <i>Str. faecalis</i> | 5.0 | | 25-50 |
| <i>Trichomonas vaginalis</i> | | | 100-> 100 |
| <i>V. cholerae</i> | | | 0.07-50 |
| <i>V. comma</i> | 2.5 | | . |

Obtained from the data of

* Waksman, Lechevalier and Harris (1949 c), Hobby, Lenert, and Dougherty (1949 a)

† Clancy (1951)

‡ Felsenfeld, Volini, Ishihara, Bachman and Young (1950 b), Ishihara and Felsenfeld (1949), Warth, Chandler and Bhargava (1950)

It is assumed that 1 Unit equals 5 μg per ml of active substance

found that the drug was able to induce resistance. Demerec and Demerec (1950) observed the step like pattern of increasing resistance in the case of *E. coli* when exposed to neomycin. Clancy (1951) noted that strains of proteus and pseudomonas showed an unusual ability to develop resistance to neomycin *in vitro*, while Hsieh and Bryson (1950) could regularly produce resistance to 200 Units per ml in *mycobacteria* by increasing the concentrations to which successive subcultures were exposed. Yegian and Vanderlinde

(1950) also failed to confirm the absence of acquired resistance in myco bacteria, as claimed by Waksman and Lechevalier (1949) Haight, Wilcox, and Finland (1952) made a study of several bacterial species from this point of view In each of them resistance to neomycin was regularly produced in contradistinction to the more erratic behaviour displayed to streptomycin Goldin (1953) also observed a stepwise increase in resistance to neomycin in *Ps aeruginosa* which, however, developed slowly compared with that to streptomycin

Cross resistance Little evidence of cross resistance was observed in *E coli* between streptomycin, chloromycetin, and neomycin (Demerec and Demerec, 1950), but strains which had been made resistant to neomycin were more resistant to streptomycin than was the original strain This finding was confirmed by Haight *et al* (1952) and elaborated to include various strains of *E coli*, *Klebs pneumoniae*, *Str pyogenes*, *Str mitis*, and *Str pneumoniae* This characteristic was not, however, evident when the organisms were tested against penicillin, the tetracyclines, chloramphenicol, hacitracin, or polymyxin B As purification of the initial product proceeded, the antibiotic was found to contain several neomycins (Swart, Hutchison, and Waksman, 1949) According to Hamre, Pansy, Lapedes, Perlman, Bayan, and Donovan (1952) both the activity and the acute toxicity of neomycin B and C were approximately equal, but little is known of the action of neomycin A

Toxicity

Unfortunately for its therapeutic effect, it was early found that neomycin, when administered repeatedly to guinea pigs experimentally infected with tuberculosis produced degenerative changes in the cortex of the kidney, loss of tubular epithelium and cellular infiltration which compressed the adjacent glomeruli and caused their degeneration (Karlson *et al*, 1950 *b*) In the rabbit, Vogel, Leopold, and Nichols (1951) found that the dry material applied to the cornea produced blepharospasm and lacrimation This latter effect might possibly have been due to impurities, as the preparation contained not more than 125 Units per mg In the cat, whereas relatively large doses of dihydrostreptomycin or prolonged treatment with this drug produced little effect on the cochlea, much smaller doses of an impure preparation of neomycin caused severe or complete degeneration of the hair cells of the organ of Corti There was, however, little effect on the vestibular function of the animal (Hawkins and Lurie, 1953) Carr, Brown, and Pfuetze (1950) probably gave the first warning of deleterious effects in man other than those on the kidneys When the drug was administered to 6 patients in doses of 0.25 to 1 G 12 hourly for 4 to 7 weeks some temporary interference in renal function was observed in all but in 4 of the patients sudden development of deafness appeared in the 4th to 6th week The deafness varied in intensity, but persisted without change even after neomycin was discontinued At much the same time Waisbren and Spink (1950 *a*) carried out clinical trials of neomycin in 63 patients with a number of diseases caused by organisms not controlled by other available drugs Most patients received 0.5 G 6 hourly intramuscularly, and this dosage was well tolerated In 24 out of 32 patients with urinary tract infections, but with no other signs of disordered renal function, fine granular casts appeared in the urine, and 6 out of 9 who did

not have albuminuria before treatment showed albuminuria during treatment. The urinary output lessened during treatment in patients with other renal signs, but most patients were able to maintain or increase the amount of urine excreted. In serial blood urea nitrogen tests carried out in 30 patients, 16 had average values of 20 mg per cent, and 11 of 20 to 40 mg per cent. Three only showed a rise in blood urea nitrogen, but in the patient in whom there was the greatest increase, the blood urea nitrogen fell again once the infection was controlled. Much more serious than these relatively transient kidney changes was the onset of deafness in 5 patients. In 1 patient impairment of hearing was observed when neomycin had been administered for 8 days. This was a case in which there was also a rise in blood urea nitrogen, and a fall in urinary output and in which casts appeared in the urine. It was, however, not until 7 days after treatment had ceased that she complained of difficulty in hearing. Subsequent serial audiograms showed that a progressive nerve deafness had taken place, but vestibular function remained normal. Fig. 7 shows how, even without a fall in urinary output, with continued parenteral administration of neomycin the patient's blood levels can rise to a height at the end of a week which may be toxic to the auditory apparatus. A second, much older, patient suffering from chronic uraemia also developed progressive nerve deafness 10 days after a 4 day course of treatment had been completed. All 5 patients who became deaf had severe renal disease with chronic nitrogen retention, or had at least shown a rise in blood urea nitrogen while being treated. In none of these patients in spite of shortness of their treatment, was there any sign of subsequent improvement in hearing, as judged by serial audiograms. Although the deafness did not appear until after treatment had ceased, this may have been fortuitous for in the patients described by Carr *et al* (1950) in whom the issue was not confused by concomitant renal disease and in whom treatment was continued over a period of some weeks, deafness occurred during treatment. This serious deterrent to the systemic use of neomycin particularly when other antibiotics were already at hand to treat most of the infections which it could control, led to its abandonment for systemic use except in desperate cases. Its use by topical application was, however, continued. In this sphere it was found to be admirable: no case of irritation from its application being seen in more than 200 cases of pyogenic infections of the skin observed by Kile, Welsh and McAfee (1950). With increasing use in this field, however, 1 case of eczematous contact sensitization was observed by Baer and Ludwig (1952).

Administration

Parenteral administration

Since deafness was such a regular occurrence when treatment was continued for long enough, little data have been forthcoming on the concentrations of neomycin obtained in body fluids after intramuscular administration. Waisbren and Spink (1950 *a*), however, assayed the concentration of neomycin found in blood serum after doses of 0.5 G had been given at 6 hourly intervals. After these repeated doses values of 10 μ g per ml were consistently found in the serum. Duncan, Clancy, Wolgamot and Beidleman (1951), with somewhat lower dosage schemes, found that after 48 to 72 hours, the

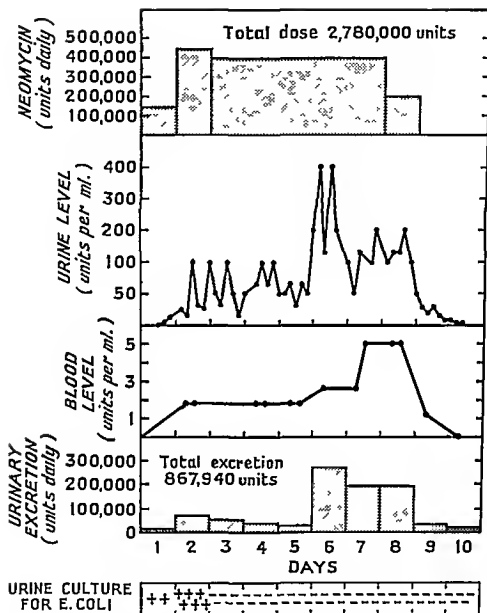


FIG 7 Levels of neomycin in serum and urine of a patient with pyelonephritis and pneumonitis treated eventually with neomycin. Though the patient remained asymptomatic throughout treatment and the urine was rendered free of blood cells and organisms it can be seen that, on a fixed dose and in spite of increased urinary excretion, the concentration of neomycin in the blood mounted steeply at the end of a week's treatment (From Duncan, Clancy, Wolgast and Beidleman, *J Amer med Ass* 1951, 145, 75)

concentrations in the blood had reached a maximum of 4 to 10 Units per ml, but if renal function was at all impaired, the blood concentrations of 26 to 410 Units per ml were reached in a patient receiving 0.5 G. 6 hourly by intramuscular injection, and these persisted in the urine for 48 to 72 hours after treatment had been stopped, the total amount excreted being somewhat less than one third of the dose administered.

Oral administration

Like bacitracin and polymyxin, neomycin is absorbed to a very small degree through the gut wall. Waelsbren and Spink (1950 a), with doses of 2 G. 6 hourly by mouth, found only 1.25 μ g per ml in the serum. Such a dosage administered by mouth could be regarded merely as a form of local treatment to the alimentary canal.

Local treatment

Drugs with some toxic effect are mostly used this way. Neomycin has accordingly been used largely for skin infections. It has usually been applied in ointments or creams at a concentration of 5 mg per G. of base without causing irritation. Local instillations of a 0.5 per cent solution of neomycin sulphate have been employed with good effect. Holzhausen (1955), Klima (1955), and Szabo (1953) used local applications of neomycin, together with bacitracin, in various surgical conditions such as compound fractures, abscesses, empyemata, and infected hands, with good effects in the great majority of cases treated.

CLINICAL TRIALS

Amoebiasis

In experimental trials *in vitro*, Felsenfeld *et al.* (1950) found that 5 strains of *E. histolytica* were inhibited by neomycin in doses of 12.5 to 50 μ g per ml. Acting on this information, McVay, Laird, and Stern (1952) isolated *Entamoeba histolytica* from a case of acute amoebic dysentery and exposed it to neomycin in an egg yolk medium. With concentrations of 100 to 1,000 Units per ml (500 to 5,000 μ g per ml) the amoebae had all disappeared from the overlay after 48 hours, and with lower concentrations of the drug they were much reduced in numbers in spite of the fact that bacteria were still present. McVay *et al.* (1952) then treated 6 patients suffering from mild amoebic colitis with 50,000 Units (0.25 G.) by mouth every 3 hours for 1 day. Following this treatment, 3 patients continued to excrete amoebae, after a short interval when their faeces were free from the parasites, but another 3 remained asymptomatic and free from amoebae for 3 months. McVay *et al.* (1952) then treated the 3 patients who had relapsed with 3 times this total dosage given over a period of 12 days at 6 hourly intervals. These 3 patients, together with 2 others who were treated along these lines, remained free of amoebae for a follow up period of 1 to 3 months. Since the time of this report little attention appeared to have been paid to the effect of neomycin on amoebiasis.

A combination of neomycin with bacitracin was given by mouth to 48 patients by Felsenfeld, Kadison, and Ishihara (1951). The patients experienced

little discomfort (1 had nausea), and the bacterial counts in the stools were reduced as early as 4 hours after the first dose. Later, stools showed that the number of viable organisms had been reduced by a factor of 10^3 to 10^6 , but after 4 to 5 days' treatment there was a slow increase in the flora. These patients, however, became free of amoebae and, during 3 to 6 months' observation, 41 had shown no relapses.

Bacterial endocarditis

The first patient with bacterial endocarditis to be treated with neomycin appears to have been a woman described by Duncan *et al* (1951). This patient developed endocarditis after having had pyelonephritis and pneumonia. Haemolytic *Staph aureus* was isolated from her blood, urine, and sputum. Penicillin, streptomycin, and chlortetracycline were administered intravenously, but the patient, who was in coma, remained so for 5 days. By this time it became known that the staphylococcus was resistant to penicillin, but sensitive to neomycin. This antibiotic was therefore administered intramuscularly at the rate of 185,000 Units per 24 hours in 6 hourly doses. The condition of the patient unfortunately remained unchanged and then deteriorated. She died on the 10th day of treatment. The blood culture, although positive 24 hours after neomycin treatment was begun, was negative on the 2 succeeding days. This patient, who was 63, was obviously not a good subject for a chemotherapeutic trial. At autopsy, although the diagnosis of bacterial endocarditis was confirmed, she was found also to have acute pericarditis, an epicardial abscess, oedema, and congestion of the brain. A more reassuring result was obtained by Kenoyer, Stone, and Levin (1952). Their patient was a young man of 20 with a past history of rheumatic heart disease which had produced both mitral stenosis and aortic insufficiency. The bacterial endocarditis was due to a *Ps aeruginosa* which was found to be insensitive to chlortetracycline, chloramphenicol, streptomycin, and oxytetracycline, but which was inhibited by $0.5 \mu\text{g}$ per ml of neomycin. When neomycin was given in doses of 0.5 G intramuscularly every 6 hours, the patient became afebrile and asymptomatic within 36 hours. All subsequent blood cultures were negative. Treatment was continued for 14 days only, and although the patient lost his hearing in the high frequency range he could still hear the spoken voice. Four months later he was well and there was no observable change in his cardiac condition. A second case of staphylococcal infection also did well with neomycin therapy (Reed and Wellman 1953). This case was a pregnant woman who developed fever, cough, and dyspnoea for which she was treated with penicillin, streptomycin, chlortetracycline, and terramycin without any effect. One week after her illness she was delivered of a stillborn infant of 8 months' gestation. At this time the heart was enlarged and there were loud systolic and diastolic murmurs at the apex and the base. A strain of *Staph aureus*, which was sensitive to between 0.1 and 0.2 μg neomycin per ml, was cultured from the blood. Accordingly, neomycin was given in doses of 0.25 G 6 hourly by intramuscular injection instead of penicillin and streptomycin, and this therapy was continued for 1 week, half this dose was given for the following 4 weeks. The patient's response was prompt: her temperature was normal in 24 hours and remained so. She was able to resume her former activity, showing no

signs of cardiac failure, although the left ventricle remained enlarged. In spite of the low dosage, however, the patient complained of buzzing in her ears by the end of the treatment, and there was loss of acuity of hearing. This loss persisted and was still present when she was examined a year later. Thus, although neomycin seems to have been remarkably successful in ridding the blood stream of both staphylococci and pseudomonas when these organisms were found to be sensitive to it, its damaging effect on hearing was evident in both cases, I having had a high dose for a short course of treatment, the other a much lower one for a more prolonged course.

Septicaemia

One case of septicaemia due to *Geotrichum* was treated by Bendove and Ashe (1952). The septicaemia apparently arose from a focus in the lungs, for these organisms were also found in the sputum. Chemotherapy was ineffective until the patient was given neomycin. Even with this drug it was 12 days before the blood cultures became sterile. After the illness the patient complained of deafness, presumably due to the neomycin. There was little reason to believe that *Geotrichum*, being a fungus, would respond to neomycin therapy, but another case had been described by Duncan *et al* (1950) in which this organism was isolated and found to be moderately sensitive to neomycin. Treatment over 4 days resulted in the urine being cleared of the *Geotrichum* as well as of Gram negative bacteria.

Meningitis

Ditkowsky, Goldman, and Goldin (1952) tested 38 pathogenic strains of pseudomonas against neomycin and found that their sensitivity *in vitro* varied between 0.2 and 50 Units per ml, the mean being 2.3 Units per ml. With this knowledge these authors felt justified in advising treatment with this antibiotic for an infant with meningitis following an operation for a meningocele. The pseudomonas was found both in the ventricular fluid and, together with *E. coli* and *Staph. aureus*, in the operation wound; it was sensitive to 3.12 μ g per ml of neomycin. After penicillin and chlortetracycline had had no beneficial effect on the infant's condition, neomycin was given in doses of 1,700 Units intramuscularly every 4 hours. After 12 days, the pseudomonas had disappeared from the wound but was still present in the cerebrospinal fluid. However, 4 days later it had disappeared from the cerebrospinal fluid as well. The child developed hydrocephalus, but this might have been due to the original congenital condition. Eight months later there were no signs to indicate that the patient had had meningitis. Another patient, a young woman, was treated by Knight, Hardy, and Negrin (1952 b). This patient contracted meningitis due to *Ps. aeruginosa* after an operation on a herniated intervertebral disc. The wound became infected with the organism, but eventually healed and the patient was discharged 8 weeks after operation. She returned 5 days later complaining of headache, stiff neck, vomiting, chills, and fever. The cerebrospinal fluid now revealed the presence of the pseudomonas. Treatment with penicillin, chloramphenicol, oxytetracycline, and streptomycin in various combinations over 6 weeks was of no avail. The patient was then given 5,000 Units of neomycin daily by intrathecal injection, together with 15,000 Units of

streptokinase and 3,750 Units of streptodornase on alternate days. Neomycin was also administered intramuscularly in doses of 120 000 Units daily. On the 9th day of treatment the cerebrospinal fluid was clear and the patient eventually made a complete recovery without sequelae.

Gastro-Intestinal antiseptics

In this field greater attention has been paid to the changes produced by neomycin in the flora of the gut. Washren and Spink (1950 a) were first impressed by the disappearance of *Proteus morgani* from the faeces of patients treated with neomycin and Poth, Martin, Fromm, Wise, and Hsiang (1951 b) made a trial of its effect on other flora. Thirty convalescent patients with normal gastro intestinal tracts were each given a low residue diet, 30 to 60 ml of castor oil and 1 G. of neomycin sulphate repeated 4 hourly for 4 doses. Unfortunately for the results of the experiment, sulphathalidine was administered simultaneously. Cultures of the stools after administration of the drugs showed no change in 2½ hours, but after 16 hours the flora had diminished in numbers and by the 35th hour only a few yeasts could be cultivated from the faeces. Neither aerobes nor anaerobes could be isolated, but on prolonged cultivation a resistant *Aerobacter aerogenes* was isolated. By the 64th hour after the first dose and 23 hours after the last, all the organisms previously found in the stools had returned and were present in similar proportions. Poth *et al* (1951 b) then carried out experiments on mice and dogs. Intraperitoneal injection of human faeces resulted in all the mice dying within 6 to 24 hours after injection, but if the faeces were injected at a time when neomycin had produced its maximum effect on the bowel flora the mice survived for periods of time related to the concentration of neomycin in the faeces. In dogs, if the abdomen were opened and filled with a 1 per cent aqueous solution of neomycin before closure, specimens collected from the colon were sterile and bowel submitted to surgical procedures healed by primary intention. Poth and his colleagues (Poth, Fromm, Martin, and Hsiang 1951 a) therefore gave neomycin with phthalylsulphathiazole as a pre operative measure for abdominal surgery. To 100 patients, over a period of 1 year these authors gave a low residue diet, 30 to 60 ml of castor oil and, after the first liquid stool, 1 G. of neomycin sulphate and 1.5 G. of phthalylsulphathiazole 4 hourly by mouth. This was continued for 3 days. At the end of this time the gut was empty and the stools cleared of bacteria. Various types of bowel resection were carried out for carcinoma colitis, closure of faecal fistulae, excision of fistula in ano, and haemorrhoidectomy. Poth *et al* (1951 a) considered this preparation ideal for securing intestinal antiseptics. Reactions occasionally occurred but they were rare. A year later Poth (1952) claimed that 350 cases had by then received neomycin by mouth some for as long as 3 months. Bacteria were eliminated from the faeces within 24 hours and although yeasts were apt to multiply, no instance of yeast infection, ulcers, or bloody diarrhoea had been seen. In 10 per cent of these cases Poth (1952) found that neomycin did not inhibit *Aerobacter aerogenes* and for this reason administered sulphathalidine together with the antibiotic in the hope that this would have some effect on the resistant bacterium.

Further investigations on the influence of neomycin on the intestinal flora

in man were carried out by Jawetz and Bierman (1952) Dearing and Needham (1953 *a*) tested the effects of neomycin alone on the bowel flora. These workers used a combination of $\frac{1}{2}$ to 1 G of neomycin daily, with 0.4 to 1.2 G of polymyxin for 3 to 6 days. In 24 to 72 hours, the coliforms, enterococci, and anaerobes had been appreciably reduced, only yeasts, staphylococci, and chromobacterium bacteroides occasionally appeared. With the lower dose patients experienced no side effects, but with higher doses nausea and vomiting occurred. When given by mouth in doses of 1 G every 4 hours, neomycin cleared the faeces of aerobic organisms within 2 to 3 days in 34 out of 37 patients suffering from various intestinal lesions. In the remaining 3 cases, however, *Str. faecalis*, *E. coli*, and a pseudomonas persisted. The presence of these organisms suggested that widespread use of the antibiotic in this manner would tend to select the resistant strains and allow them to persist. In another endeavour to rid the faeces of every type of bacterium, Dearing and Needham (1953 *b*), after a low residue diet and a mild saline purge, combined the administration of 0.25 G of oxytetracycline 4 times a day together with neomycin. With this therapy both aerobic and anaerobic organisms disappeared. No resistant staphylococci were seen but there were some preliminary experiments which indicated that such organisms could develop after 5 or more days' incubation. Dearing and Needham (1953 *b*) recommended that the dose of neomycin should be raised to 1.5 G 4 hourly on the 2nd day, in order to sterilize the gut before operation. Following these studies Pettet, Judd, and Dearing (1955) observed the effect of pre-operative preparation of patients with neomycin and with neomycin combined with oxytetracycline. A mild purge was again given and the dose of neomycin remained the same. Seventy-two patients were treated with neomycin and 71 with the 2 antibiotics. The results did not seem to be very different in the two series except for the complication of staphylococcal enterocolitis. This appeared in 4 patients on the oxytetracycline-neomycin regime and 3 of these died. Pettet *et al* (1955) drew attention again to the likelihood of strains resistant to neomycin appearing should this treatment be continued for long periods.

Other workers who made use of neomycin in the pre-operative preparation of the gut were Mann, Schumer, and Tomusk (1954), Moritsch (1955), and Rowlands and Scorer (1955). With preparations similar to those used by Pettet *et al* (1955), Mann *et al* (1954) operated on their patients within 24 hours of their receiving neomycin. At operation the large intestine was found to be contracted and free of aerobes, and there was no sign of irritation of the mucosa. The intestinal contents remained sterile for an average of $5\frac{1}{2}$ days after operation, thus obviating the necessity of treating patients chemotherapeutically after operation. In fact in 17 patients who did receive post-operative chemotherapy with several antibiotics, complications occurred in 5, 2 of these being due to staphylococcal enterocolitis. In an attempt to ensure the control of the staphylococcus, Moritsch (1955) prepared 11 of his patients with a combination of neomycin and bacitracin (discussed on p. 150). With a dose of 0.5 G of each of these antibiotics 3 times a day for 3 days, it was possible to rid the intestine of cocci, enterococci, *E. coli*, and clostridia, but with a lower dose the results were uncertain. Moritsch (1955) drew attention to the poor bacteriological results obtained when there was stenosis of the bowel. Rowlands and Scorer (1955) compared the effect of 2 different

dosage schemes with neomycin alone used in the pre operative preparation for major bowel surgery. Fourteen patients received 1 G hourly for 4 doses and then 1 G 4 hourly until the end of the 24 hours. Sterilization of the bowel contents occurred more frequently in the second group, but the clinical results were similar in both. No operation was fatal and there was no intra peritoneal infection or wound sepsis. No neomycin resistant organisms were found. Still further work was done in this field by Milberg, Kamens, Ripstein, and Banowitch (1955). These authors examined the flora obtained in rectal swabs from patients on admission to hospital and tested the sensitivity of the organisms found to 10 different antibiotics by the disk plate method. It was found that the highest percentage of strains (86 per cent) were inhibited by neomycin or chloramphenicol. For reasons unexplained these workers then compared the effects of 3 antibiotic schemes in the pre operative preparation of 100 patients. A cathartic was first given and the antibiotics were then administered for 2 to 4 days accompanied by enemas twice daily. The measures taken and the resulting effects on the bowel cultures were as follows:

| | No. cases | Bowel cultures sterile (cases) |
|--|-----------|-----------------------------------|
| Neomycin 0.5 G 6 hourly for 2 days | 10 | 0 |
| Chlortetracycline 0.75 G 6 hourly for 4 days | 22 | 0 |
| Neomycin 3.5 G + Oxytetracycline 0.25 G } 6 hourly for 2 days | 50 | 30 |

From a study of the remaining flora it was found that *E. coli*, proteus, and *Staph. aureus* were most commonly found, but it is not stated in which group these different organisms were most commonly found, or whether they were sensitive to any available antibiotics.

Combinations of neomycin with erythromycin, oxytetracycline, or carbomycin have also been tried by Prigot, Shidlovsky, Turell, and Marmell (1955 *a* and *b*). Adult patients with no signs of gastro intestinal lesions received 4 G of neomycin daily in equally divided doses for 1 or 2 days or the same doses of neomycin and erythromycin. No cathartics or bowel washouts were given. Neomycin alone reduced the number of Gram negative aerobes in the stools within 24 hours, and Gram negative anaerobes and aerobes within 48 hours, but there was no appreciable reduction in the number of Gram positive organisms. With erythromycin the latter organisms were also reduced in number. The same dose of neomycin and 0.2 G of oxytetracycline, or 0.5 G of carbomycin, 4 times a day, produced similar results to those obtained with erythromycin. In each set of investigations stress was laid on the reduction in the number of enterococci produced by the combined treatment. Further experiences of Poth (1957) led him to consider a combination of neomycin with bacitracin as the nearest approach to the ideal intestinal antiseptic, provided bacitracin was continued for 3 days longer than neomycin. He pointed out, however, that after operation, during the stage of paralytic ileus, administration of drugs by mouth could have no effect on the flora of the gut. If there was any risk of contamination of the peritoneal cavity, therefore, he placed at operation 200 ml of a 0.5 per cent solution of neomycin containing 500 Units of bacitracin within it and repeated the procedure at the end of the operation when the first solution was nearly all lost. When there was a question of resection of the intestine,

the solution was injected through the abdominal wall pre operatively to ensure the minimum of viable bacteria in the field of operation 30 minutes later After 16 years of practising one form or another of intestinal antiseptics, Poth was enthusiastic about this method of treatment and claimed that with it convalescence after extensive resection and primary anastomosis closely paralleled that from a clean operation such as herniorrhaphy When bacitracin was considered too expensive, phthalylsulphathiazole provided a good substitute Introduction of neomycin into the peritoneal cavity, however, carries with it the risk of inducing apnoea Middleton, Morgan and Moyers (1957) described the case of a man of 75 with a gunshot wound of the abdomen involving much of the intestines After partial resection of the large intestine and closure of many perforations of the small intestine a solution containing 2 G of neomycin was instilled into the peritoneal cavity Fifteen minutes after the instillation, when the patient was reacting well with spontaneous breathing and wrinkling of his forehead his blood pressure fell from 100 to 10 mm Hg and he ceased to breathe By means of artificial ventilation, a levarterenol drip, and injections of atropine sulphate and neostigmine, his breathing was restored but he died 23 hours later in a shock like state at a time when the levarterenol drip could not be started again quickly Other instances, not all fatal, have been described by Webber (1957) Two infants died but adults who had 3 and 5 G of neomycin placed in their peritoneal cavities survived after suitable restorative measures It would seem advisable, therefore to use neomycin in the peritoneal cavity with considerable caution and not to exceed the amount used with impunity by Poth

Should neomycin or any combination of neomycin and other antibiotics active against Gram positive organisms be used extensively, the possibility of resistant strains arising and spreading to other patients cannot be disregarded

Diarrhoeal diseases

Diarrhoea due to salmonellae or shigellae in 25 young children was treated by De Leon (1955) with 50 mg of neomycin per kg of body weight per day divided into 4 hourly doses The onset of the diarrhoea was sudden and there were bloody and mucoid stools In no case was treatment begun later than on the 3rd or 4th day of the illness With the treatment described, 21 of the children had recovered clinically and bacteriologically within 4 days No reason was given to explain why 4 children did not respond As the infection in 1 case was due to *Salmonella typhi* and in 3 to shigellae, the cause would appear not to have been due to insusceptible bacteria A series of 53 children under 1 year of age with diarrhoea was treated by Kadison and Borovsky (1951) Acting on the observation made by Young, Yoshimura, and Felsenfeld (1950) that neomycin and bacitracin were synergistic in their action Kadison and Borovsky gave these children tablets containing 50 mg of neomycin and 2 000 Units of bacitracin 6 hourly The infecting organisms were not known but a few children were found to have amoebae in their stools These latter cases received twice the routine dose The average duration of the diarrhoea in the children receiving the 2 antibiotics was 3.3 days whereas it was 6.4 days in those not receiving the antibiotics In view of the doubtful origin of these diarrhoeas there seems to have been little justification for using the 2

antibiotics, except that if one of them did not act against the causal bacterium, it was possible that the other would. In other cases of diarrhoea treated with neomycin, *E. coli* seems to have been mainly responsible. Gorzynski and Neter (1953 a) drew attention to the fact that strains of *E. coli* belonging to sero groups O 111, O 55, and O 26 seemed to be associated with epidemic and sporadic diarrhoea in infants. These authors tested 29 strains belonging to these groups and found all of them to be sensitive to neomycin *in vitro* in concentrations of 1.5 to 50 μg per ml, 7 of these strains grew profusely in 100 000 μg per ml of streptomycin. On passage through increasing concentrations of neomycin and streptomycin, resistance to the drugs developed, but not so readily to neomycin as to streptomycin. An epidemic which was thought to be due to *E. coli* O 111 broke out in the Children's Hospital Columbus, Ohio, and was described by Wheeler and Wainerman (1954). A dosage of 50 mg of neomycin per kg per day was administered by mouth together with 35 mg of chloramphenicol per kg per day, and vitamin K. After this regime was instituted no further deaths occurred in the paediatric unit where these children were being nursed, but relapses were frequent. The authors note that there were many resistant organisms in the stools after chloramphenicol had been given and they therefore considered neomycin preferable. Another epidemic in which *E. coli* O 111 B₄ was isolated from the stools of the patients was described by Stulberg, Zuelzer, and Nolke (1954). This epidemic occurred in a nursery for newly born infants. *E. coli* O 111 B₄ in nearly pure culture was isolated from 24 of these infants, but was not found in infants that were isolated and remained well. Dehydration, lethargy, an unstable temperature, loss of weight, anorexia, and vomiting accompanied the diarrhoea in half the children. Chemotherapy was limited to neomycin alone, which eliminated the organisms from the stools, and this was shortly followed by cessation of symptoms in the majority of cases. Unfortunately 3 patients died. Yet another outbreak of gastro enteritis due to *E. coli* O 111, this time in the Children's Hospital, Birmingham, yielded to neomycin (Rogers, Benson, Foster, Jones, Butler, and Williams, 1956). One hundred and one babies were either severely ill or carriers of this type of *E. coli*, which was insensitive to chloramphenicol, chlortetracycline, oxytetracycline, and several sulphonamides including phthalylsulphacetamide. Neomycin sulphate was made up in a fluid mixture with kaolin and suspended in methyl cellulose, the daily dose being 20 mg per lb of body weight, which was continued for 4 to 13 days. In most infants there was a rapid disappearance of the specific serotype of *E. coli* from the faeces together with an early cessation of symptoms or a failure to develop gastro enteritis in those who were carriers. There were 4 deaths—from a generalized staphylococcal infection, from meningitis, and in 2 who died very shortly after treatment was commenced. In the others both bacteriological and clinical response was good, relapses occurring in not more than 18 per cent. *E. coli* was again thought to be responsible for an outbreak of epidemic diarrhoea in infants in the Children's Hospital, Cincinnati. This time a different serotype, *E. coli* O 127 B₈ was implicated (Cooper, Walters, Keller, Sutherland, and Wiseman, 1955). One hundred and fifteen of the infants and children had diarrhoea at some time during their stay in hospital, and from 44 of them this strain of *E. coli* was isolated. The onset of the diarrhoea was sudden, with much abdominal distension and a peculiar pungent and musty odour of the faeces. Neomycin was

given by mouth to 36 of these 44 cases in doses of 6 to 80 mg per kg of body weight daily and continued for 2 to 26 days. With such a wide range of dosage it is not surprising to find that the *E. coli* was still present in 12 patients at the end of treatment. Moreover, 4 of these children died, and also 1 in whom this strain of *E. coli* was not detected. Nevertheless only 2 of the survivors had a recurrence of their symptoms. As a prophylactic measure, neomycin was administered to every infant in the wards affected by the epidemic in doses of 40 to 50 mg per kg of body weight daily. This measure was followed both by a decrease in the incidence of the diarrhoea and in the incidence of *E. coli* O 127 B₃ in cultures from rectal swabs. This strain was found to be inhibited also by other antibiotics, by polymyxin, neomycin, chloramphenicol, tetracycline, and oxytetracycline in descending order of efficacy.

Liver disease

On the assumption that toxic nitrogenous substances, particularly ammonia and amines, responsible for hepatic coma are derived from the small intestine, neomycin has been employed to counteract their effect. In the cirrhotic patient the small intestine, contrary to that of the normal person, harbours an abundance of organisms, particularly coliforms, which break down proteins and amino acids with the liberation of ammonia and amines which are then liberated into the blood stream without change by the diseased liver. Because of its bactericidal powers and of its small absorption from the gastro intestinal canal neomycin was considered a suitable agent for preventing their formation. Twenty patients, 12 in acute hepatic coma and 8 suffering from chronic portal systemic encephalopathy, were given 4 to 10 G daily by mouth by Dawson, McLaren, and Sherlock (1957), together with complete withdrawal of protein, and the giving of enemata and blood transfusions in acute cases. A limited constant daily protein intake was prescribed for chronic cases. Under treatment the fasting arterial blood ammonia level fell gradually in the chronic patients with a disappearance of fetor hepaticus and an improvement in the electroencephalogram. The effect on the stool flora was, however, variable and could not be related to the clinical benefit. Acute cases improved from deep coma to mild confusion or to normal mentality. In the more chronic condition the benefits from neomycin could only be depended on while the drug was being administered—symptoms returned in 2 patients when it was discontinued after 4 and 10 months. Similar conclusions were reached by Fisher and Faloan (1957), the venous ammonia level was reduced to normal in 11 out of 12 patients with cirrhosis and neurological and mental symptoms improved. Stool cultures, however, became sterile or grew only urease negative organisms. Like Dawson *et al.*, Fisher and Faloan found these changes depended on the continuation of administration of neomycin, the blood ammonia level rising promptly when it was discontinued.

Urinary infections

Forty of the 63 patients treated by Waisbren and Spink (1950a) were suffering from infections of the genito-urinary system. Most were chronically infected and the test of the antibiotic was therefore a severe one. Waisbren and Spink (1950a) deliberately chose cases where *B. proteus* was cultured from the urine, as this organism is particularly difficult to eradicate by other

antibiotics, and bacteriological studies gave some hope that it would respond to neomycin. Seventeen patients were therefore chosen and treated with 0.5 G of neomycin 6 hourly by intramuscular injection for 3 to 8 days. The urine was sterilized in 13 of these, but in 3 *Ps aeruginosa* was subsequently isolated, while in 1, *B proteus* persisted. In this last case the presence of foreign bodies in the shape of bilateral ureteral catheters militated against the elimination of infection. From the urine of another 11 patients, *Ps aeruginosa* was cultivated, and in 9 of these the urine was sterilized by neomycin even when other Gram negative organisms were present in addition to the *Ps aeruginosa*. Four cases of infection with *E coli* were successfully treated. In 8 patients infected with *A aerogenes* the urine was sterilized, but *Ps aeruginosa* appeared later in 1 of these cases and γ streptococci in another. Another series of patients described in detail were those of Duncan *et al* (1951). These authors, like Waisbren and Spink (1950 a), chose cases in which the infecting organisms were insensitive to other therapeutic agents but were sensitive to neomycin as shown by the filter paper disk technique of Bondi, Spaulding Smith, and Dietz (1947). These cases had pyelonephritis, prostatitis, and cystitis, or cystitis with calculus formation. The infecting organisms found in the urine of these patients were *A aerogenes*, *E coli*, paracolonic bacillus, coliforms, coliforms together with *Ps aeruginosa* and, in 1 case, a staphylococcus. The treatment varied somewhat. In the 1st case the doses were initially 20 000 Units 4 hourly, but they were soon raised to 40 000, 80 000, and finally 100 000 Units 6 hourly. This latter dose was adopted for the next 3 patients, but was subsequently reduced to between 30 000 and 50 000 Units 6 hourly. These lower doses appeared to be as effective as the higher ones except in 2 patients, one who in addition to the kidney disease, had pyaemia and staphylococcal endocarditis and for whom no dose was likely to be curative, and another with calculi in the kidneys. Irrespective of the dose up to 100 000 Units 6 hourly, neomycin was not able to clear the urine of pus or of streptococci or *Ps aeruginosa*. In contradistinction to the findings reported by Waisbren and Spink (1950 a), Duncan *et al* (1951) found that most strains of proteus and of *Ps aeruginosa* showed increasing resistance to neomycin while under treatment with this drug. In spite of this drawback, it was the cases with urinary tract infections who most consistently improved under neomycin treatment (Wolgast and Duncan, 1950).

Other workers who used neomycin in the treatment of urinary tract infections were Nesbit, Dodson, and Mackinnon (1952), Ferguson and Carron (1954), who confined their studies to the treatment of non specific urethritis, Korner and Jepsen (1955) and Cox, Soanes, and Lowry (1955). Nesbit *et al* (1952) gave a low dose of 0.25 G intramuscularly every 6 hours for a maximum of 5 days. In 12 out of 18 cases the urine was sterile by the end of treatment and remained so in 8 of these cases for 1 week to 9 months. In 2 cases a different set of organisms appeared during treatment, and no change was observed in 3 cases. (In the 18th case, no organisms were found in the smear, and cultures were not done.) In the cases which responded the result was prompt, and pyuria had markedly diminished in 24 to 48 hours. With this small dose there was little evidence of toxicity, granular casts appeared temporarily in 3 patients but these disappeared at the end of treatment. One patient suffered from severe nausea and vomiting, and the neomycin was discontinued after 2 G had been given, but there was no mention of deafness.

In another patient with chronic pyelonephritis and pyelo ureterocystitis, small doses of neomycin were administered at varying intervals over a period of a year without producing ill effects and with marked relief of symptoms. This patient was described by Cox *et al* (1955). The infecting organism was *A. aerogenes* which persisted in spite of treatment with various other antibiotics. It was, however, inhibited by $2\text{ }\mu\text{g}$ per ml of neomycin and also by streptomycin. A course of neomycin in doses of 0.5 G twice a day, and dihydrostreptomycin with streptomycin in doses of 1 G each daily, was given for 1 week. Within 48 hours there was no microscopic pyuria and the pain had subsided. Neomycin was continued in doses of 0.25 G twice a week and at a later stage once a week for 6 weeks. In spite of this rather lengthy treatment, recurrence took place 17 days after neomycin treatment was stopped, but this recurrence was cleared up after 1 dose of 0.5 G. In the following 10 months 6 mild recurrences were treated similarly, the organism on each occasion being *A. aerogenes*. One dose of 0.25 G of neomycin cleared the pyuria and sterilized the urine each time. The *A. aerogenes* remained moderately sensitive to neomycin during this therapy. When cystoscopy and retrograde pyelography were carried out the cystic lesions were found to be much reduced, the blood urea nitrogen was normal. An audiogram revealed a 12 to 15 per cent bilateral loss of hearing for high tones but hearing of the spoken voice was not impaired. How useful neomycin can be in refractory infections of the urinary tract was brought out by Niebel and Rosenberg (1956). The 31 cases they chose to treat were all severe, the responsible organisms being Gram negative and resistant to other agents. Some had obstructive lesions and in others there was renal damage to a varying degree. Niebel and Rosenberg prescribed 0.5 G every 12 hours for 5 days, finding this to be a relatively safe dose. The drug was well tolerated without gross neurotoxic effects or side effects such as diarrhoea, itching, dizziness, headache, or nausea. Transient proteinuria with granular casts appeared in 5 patients, but did not necessitate discontinuing treatment. Nevertheless with this dose the urine in each patient returned to normal either during treatment or immediately after it.

With the increasing resistance shown by bacteria to antibiotics in the past few years it is satisfactory to know from Roantree and Rantz (1956) that among 1,698 strains isolated during the 2 years of 1954 and 1955 neomycin was still the most active antibiotic against *E. coli*, and the paracolon bacillus, proteus and aerobacter groups of organisms. *Pseudomonas*, however, was seldom inhibited by $10\text{ }\mu\text{g}$ per ml. Though it was not possible to expect permanent cure in patients suffering from chronic and obstructive conditions yet, acting on their knowledge of the susceptibility of the infecting organisms, Roantree and Rantz treated only those infections where the bacteria were sensitive to neomycin. With intramuscular injections seldom amounting to more than 1 G daily, the first cultures after treatment showed no growth in 15 of their 20 patients, *Ps. aeruginosa* persisting or appearing in the others. Fall in temperature and relief of symptoms were usually prompt. In these patients there were no signs of toxicity and proteinuria appeared in 1 only of the 11 patients who did not give evidence of it before treatment. Thus, with a dose limited to not more than 1 G a day for 5 days, these two pairs of workers demonstrated both the small risk of administering neomycin and its rapid and powerful effect on susceptible infections.

Cystitis and non specific urethritis

Local applications of neomycin were used for the treatment of non specific urethritis and cystitis by Ferguson and Carron (1954) and Korner and Jepsen (1955). Ferguson and Carron instilled 10 ml of a 0.5 per cent solution of neomycin sulphate into the urethra in 65 patients who had not responded to other chemotherapy. The solution was kept in the urethra for 20 minutes and the process repeated if necessary up to 7 times. Fifty-nine of these cases showed definite improvement or complete elimination of the discharge. Korner and Jepsen (1955) used a combination of neomycin with bacitracin for instillations into the bladders of 12 patients confined to bed with chronic cystitis. The instillations, however, were not very effective, and intramuscular injections of 0.25 G of neomycin twice daily were given for 5 days. In 5 out of 7 patients so treated the urine became sterile and the symptoms disappeared.

Infections of the skin, and burns

Kile, Welsh, and McAfee (1950) treated skin infections by topical application of 5 mg of neomycin per G in ointment or in a water miscible base. More than 200 cases of impetigo, furunculosis, sycosis, and infectious eczematoid dermatitis were so treated. No case of irritation was found to be due to neomycin, and in the majority of cases the results were considered to be superior to those achieved by any previous preparation. When 605 cases had been treated, Kile, Rockwell, and Schwarz (1952) made another report. Similar preparations were applied 3 to 5 times a day in a series of skin conditions which now included otitis externa, herpes simplex, secondary pyogenic infections, hydradenitis suppurativa, non-specific granuloma, acne varioliformis, perleche, stasis ulcers, burns, infected sebaceous cysts, and dermatitis repens. It was not to be expected that uniformly successful results would be achieved. The number of cases showing marked improvement was 217, and most of these were infective dermatitis, impetigo, and secondary pyogenic infection. When bacteriological examinations were carried out on a sample of these cases, it was found that marked improvement occurred in infections due to haemolytic *Staph aureus*, haemolytic streptococci, staphylococci, and *Ps aeruginosa* in that order of frequency. Infection with *Pseudomonas* alone was not improved by treatment. Kile (1953) then tried using a 0.5 per cent preparation of neomycin together with hydrocortisone as a prophylactic measure against infection in skin conditions. The best results were obtained in cases obviously infected with bacteria. The majority of cases with contact dermatitis, atopic dermatitis, infectious eczematoid dermatitis, pruritus ani (1 case), dysidrosis, and seborrhoeic dermatitis did well, but no improvement resulted from the application of either neomycin or cortisone in cases of lichen simplex chronicus, lupus erythematosus, psoriasis, alopecia, lichen planus, pilonidal cyst, perleche, stasis dermatitis, pustular eruption in palms and toes, Fox Fordyce disease, lichen sclerosis and atrophicus, insect bite, sarcoid and nuchal dermatitis. Livingood, King, Stevenson, and Mullins (1952) also used neomycin sulphate in a concentration of 5 mg per G of petrolatum and lanolin base or in concentrations of 1 to 5 mg per ml in aqueous solution used as a compress or soak for pyogenic infections of the skin or for burns. Curiously enough in nearly 200 lesions in which the infecting organisms were

tested for their sensitivity to neomycin, the majority of staphylococci were inhibited by 0.4 μg per ml or less, but very few haemolytic streptococci and no proteus or pseudomonas were so sensitive. Like Kile (1953), these authors found that the majority of lesions with purulent infections responded to treatment. In the treatment of burns, Lavingood *et al* (1952) did not find neomycin superior to other antibiotics in controlling infections due to Gram positive organisms, but found it preferable for cases infected with proteus and pseudomonas. A number of other workers have published the results of their experiences with neomycin in the treatment of skin infections, among them being Forbes (1953), Church (1954), Caldwell (1954), Peterkin (1954). The results of trials with a combination of neomycin and bacitracin were published by Gade, Korner, and Sylvest (1953), Montgomery and Montgomery (1953), Perdrup (1953), and Rattner and Rodin (1952). A combination of neomycin with gramicidin was used by Leshe and Harvey (1954). It is with difficulty that any distinction can be made in these reports between the relative merits of neomycin alone or in combination with another antibiotic. That neomycin is not free from side reactions has been demonstrated by Peterkin (1954) and Goldberg (1957), and it does not prevent the supervention of moniliasis on rare occasions (Forbes, 1953). In 1953-4 there was little to choose between the effects of neomycin and chlortetracycline (Caldwell, 1954), but the much more widespread use of chlortetracycline since that time may have weighted the scales in favour of neomycin. When the reports of neomycin used in combination with another antibiotic are considered, the combination of neomycin with bacitracin seems to be superior to neomycin with gramicidin. The former combination has not been applied very widely, but it has the advantage of being effective against a wide range of bacteria and of killing rather than merely inhibiting the bacteria in attainable concentrations. Response with this combination should be rapid and should therefore provide less opportunity for sensitization to occur than in the case of prolonged treatment.

Infections of the eyes

Neomycin sulphate in a concentration of 5 mg per G of ointment base was used by Lopez (1954). This author treated 36 cases with superficial infections of the eyes. When these were acute and bacterial in origin, improvement was observed in 2 to 5 days, but catarrhal conjunctivitis, not associated with bacteria, did not respond to treatment and continued for over a week. Chronic conditions did not respond as rapidly as the acute bacterial infections, but, even so, when bacteria were isolated, and they were mostly staphylococci, improvement occurred in time.

Infections of the ear and nose

A series of infants and children with otitis media, mainly caused by *Ps aeruginosa*, were treated with local applications of neomycin by Lazar, Goldin, and Auerbach (1952). In association with otitis media, these children were also suffering from infections of the lungs, skin, or urinary tract due to the same organism. Neomycin was therefore also administered in doses of 1,500 Units intramuscularly every 4 hours. Good results were reported and there were no ill effects. Local applications of the drug in a concentration of 200 Units per ml were also used with beneficial effects. At a later date, Lazar

and Goldin (1955) relied only on local applications in which neomycin was combined with gramicidin, the antihistaminic thonzylamine hydrochloride and a decongestant, phenylephrine. All these constituents were incorporated with a cationic surface active agent, thonzonium bromide, in an aqueous solution. The preparation was found to have bactericidal powers *in vitro* against a number of different species, both Gram positive and Gram negative, without being irritating to inflamed tissues. It was used as a spray for 124 patients with nasal conditions such as rhinitis, maxillary sinusitis, chronic nasopharyngeal catarrh, coryza, 'influenza', and hypertrophic rhinitis. From at least half of these conditions a bacterium was isolated. The relief experienced by 113 of the patients after application of this preparation could hardly have been altogether due to the decongestant action of phenylephrine. Two hundred and eighty two patients with various types of otitis externa and media were also treated, of whom 88.7 per cent experienced relief or disappearance of signs of infection.

CONCLUSION

All these antibiotics with some toxic effects have two great advantages: they are bactericidal and they do not appear to harm superficial tissues or the linings of cavities. One is tempted to advise that their use be limited to local application and oral administration, but when all other likely agents have been given a fair trial and the patient is in danger of losing his life, the consideration of whether the kidneys or the hearing may suffer from the drug does not weigh so heavily. The drugs of this group which have shown that they can be used with caution for a short time parenterally are bacitracin, polymyxin B or E, and neomycin. The doses advised are as follows:

| | |
|-------------|--|
| Bacitracin | 80,000 Units per 24 hours not more than 100,000 Units per 24 hours |
| Polymyxin E | 2.5 mg per kg per 24 hours, not more than 4.8 mg per kg per 24 hours |
| Neomycin | 0.25 G 6 hourly, not more than 0.5 G 6 hourly |

CHAPTER 6

ANTIBIOTICS ACTIVE AGAINST PROTOZOA, FUNGI, OR NEOPLASTIC CELLS

FUMAGILLIN, ANISOMYCIN, PUROMYCIN, NISTATIN, ACTIDIONE,
THE AMPHOTERICINS, THE ACTINOMYCINS, SARKOMYCIN,
AMICETIN

FUMAGILLIN

PREPARED from *Aspergillus fumigatus* by Hanson and Eble (1949), fumagillin was found to be amoebicidal in very high dilutions, but it had little antibacterial activity. It had been obtained in a crystalline form by 1951 and was considered to be a monobasic acid (Hrenoff and Nakamura, 1951). It was found to kill amoebae in dilutions of 1:10 million and even at concentrations of 1:130 million (McCowen, Callender, and Lewis, 1951). No associated bacterial growth was influenced by the antibiotic. Trials *in vitro* by Balamuth (1953) with other amoebae—*Entamoeba coli*, *Dientamoeba fragilis*, and *Endolimax nana*—showed that fumagillin was the only amoebicide as effective as emetine against these protozoa. Killough, Magill, and Smith (1952) also observed an action on *E. coli*, *Giardia lamblia*, *Chilomastix mesnili*, and *Iodamoeba buetschlii*.

Trials *in vivo* in rats, rabbits, and macaque monkeys infected with *E. histolytica* showed that the stools of all these animals were rapidly cleared of amoebae, but in the monkeys the amoebae reappeared within 4 to 12 weeks of treatment (McCowen *et al.*, 1951; Hrenoff and Nakamura, 1951). Fumagillin was without effect on *Spirochaeta novyi*, *Trypanosoma gambiense*, and *Trypanosoma equiperdum* in mice (McCowen *et al.*, 1951), nor did it affect *Schistosoma haematobium*, *Ascaris lumbricoides*, *Ankylostoma duodenale*, *Enterobius vermicularis*, or *Hymenolepis nana* (Killough *et al.*, 1952).

Its toxicity, as judged by its effect on mice, was slight, the LD₅₀ by subcutaneous injection being 800 mg per kg and, when given by mouth, 2 G per kg were tolerated. In man—10 volunteer students—doses of 10 mg, increased gradually to 100 mg, daily were tested. Anorexia was produced when doses of 30 mg were reached, nausea and abdominal discomfort with 70 mg, and mild vertigo in most subjects when the dose exceeded 60 mg (McHardy, Bechtold, Welch, and Browne, 1953).

CLINICAL TRIALS

These began when Killough *et al.* (1952) treated 22 male patients in the United States Naval Medical Research Unit at Cairo. Each patient received between 5 and 50 mg daily in divided doses for 2 weeks. In patients with

mild symptoms due to *E. histolytica*, the organism disappeared from the stools within 48 hours, but the follow up was not long enough to make sure that no relapses occurred. Few ill effects from the drug were noted. Two patients taking 50 mg daily complained of dizziness and 4 others lost their appetite but had no nausea. Another trial of 134 ambulatory patients with *E. histolytica*, showing cysts or trophozoites in their stools, was conducted by McHardy *et al* (1953). The patients were considered to have recovered when their stools were free from *E. histolytica* when examined 3 times at monthly intervals, and when there was radiological evidence of the healing of ulcers. On this criterion 64 cases recovered. Of these, there were 12 who had had acute ulcerative colitis and 18 who had had the chronic illness. In some 475 other cases treated by various workers¹ in doses which amounted to 20 to 200 mg daily, therapeutic results did not differ widely, except in the cases of Elsdon Dew, Wilmot and Armstrong (1953), but the unpleasant side effects increased with the dose. The duration of treatment lasted usually between 10 and 14 days but, on occasion, courses were repeated when *E. histolytica* reappeared in the stools. With careful attention to the patients' treatment it seems possible to rid the stools of these protozoa in 90 per cent or more of cases (McHardy, 1954; Portilla, Becerra, and Ruiloba, 1954; Schindel, 1954; and Mentasti and Grassi, 1955). Nevertheless it is well to bear in mind that doses of over 10 mg 3 times a day are liable to produce malaise, dermatitis which may not be severe in itself but which leads to desquamation of hands and feet, abdominal pain, anorexia, nausea, and/or nervousness leading to loss of sleep.

Fumagillin with other antibiotics

Fumagillin and the tetracyclines

Results of treatment of several hundred patients in a United States Public Health Service hospital were reported by Terry and Spicknall (1955). These authors had come to the conclusion that the best results were obtained with one of the tetracyclines which can only be depended on to eradicate amoebae indirectly, together with an amoebicide, emetine hydrochloride being the drug most favoured. Nevertheless fumagillin was used in 17 asymptomatic carriers and 13 patients with symptoms, the dose being 10 mg 3 times daily for 14 days. The stools became negative in all patients and remained so for 16 weeks in 15 patients for longer still. With the low dosage used, no serious ill effects were produced by the treatment. Shaffer (1955*b*) also used both antibiotics together in treating patients with acute dysentery and found that, although tetracycline given for 10 days cleared the diarrhoea in a shorter time than tetracycline together with fumagillin, the later combination left fewer cysts in the faeces after treatment. No recurrences had occurred up till the time of reporting.

Fumagillin with erythromycin

Jung, Garcia Laverde, and Katz (1955) treated Panamanian school children with one or other or both of these antibiotics and compared the results.

¹ Black, Terry and Spicknall (1954); Elsdon Dew, Wilmot and Armstrong (1953); Killough and Magill (1954); McHardy (1954); McQuiddy (1954); Mentasti and Grassi (1955); Pinto and Pezzullo (1955); Portilla, Becerra and Ruiloba (1954); Schindel (1954); Theodoris and Kessel (1953).

With erythromycin (100 mg daily for 7 days) *E. histolytica* disappeared in 33 out of 39 patients, and when the 2 drugs were used together *E. histolytica* disappeared in 62 out of 63 patients. These results merely serve to show that a combination of the 2 antibiotics had as good an effect as erythromycin alone. It does not give an indication of whether the infection was eradicated over a period of some months more effectively by the 2 antibiotics than by 1 antibiotic alone. Anderson, Nelson, Carbone, and Diaz (1955) also found that in a small number of patients the use of erythromycin together with fumagillin made little difference to the outcome of treatment, although the results slightly favoured erythromycin.

ANISOMYCIN

This antibiotic was announced first by Sobin and Tanner (1954). The activity *in vitro* of anisomycin was tested by Lynch, English, Bauck, and Deligianis (1954). Amongst 46 different micro organisms against which the antibiotic was tested, those of greatest interest in human disease were *E. histolytica*, which was inhibited by 1.56 μg per ml, *T. vaginalis* and *T. foetus*, which were inhibited by 1.56 to 3.21 μg per ml, and *Candida albicans*, which was inhibited by 1.56 to 12.5 μg per ml. Bacteria, including *Staph. aureus*, *Str. faecalis*, mycobacteria and Gram negative organisms, all required more than 100 μg per ml for inhibition, whereas pathogenic fungi needed 100 to more than 500 μg per ml. Although bacteria were beyond the range of the effective activity of anisomycin, the sensitivity of trichomonads and even of *E. histolytica* to this drug was many times greater than to the tetracyclines, erythromycin, or fumagillin, or to other drugs. Anisomycin was even found to kill *T. vaginalis* at a concentration of 3.12 μg per ml, and remained lethal at this concentration in spite of variations in the size of the inoculum (Lynch, Holley, and Salmirs, 1955).

Protective trials in mice infected subcutaneously with *Trichomonas foetus* showed that the antibiotic was capable of clearing the animals of infection provided that treatment was begun immediately after infection and continued for several days (Lynch, English, Morrison, and Maves, 1954). The toxicity of the antibiotic was not high, the LD_{50} for mice by oral or intravenous administration being 140 to 148 mg per kg of body weight. Rats showed no chronic toxicity when fed up to 50 parts per million of anisomycin for 33 weeks, and monkeys tolerated up to 64 mg per kg daily by mouth over more than 6 months. The only ill effect observed was occasional vomiting and diarrhoea in the group of monkeys receiving the highest dose—64 mg per kg (Gardocki, Timmens, Wilson, Sodergren, Hettlinger, and Pan, 1955).

The only clinical trial brought to the author's notice was that of Armstrong and Santa Maria (1955). These workers treated 88 married women suffering from leucorrhoea or pruritus vulvae due to trichomonads. Two 1 mg tablets of anisomycin were introduced 3 times weekly into the posterior fornix and directed to the right and left of the vaginal vault. The procedure was continued for 2 weeks. During this time, and 1 week after the final application, vaginal smears were examined for trichomonads. By this time results could be summarized as follows:

| | No of cases | No of cases at time from treatment | | | |
|---|-------------|------------------------------------|------------------------|------------------------|-------------------------|
| | | On 5th day | 3 days after treatment | 1 week after treatment | After next menstruation |
| 2 specimens free of trichomonads | 77 | 57 | 57 | 29 | 33 |
| A reduction of trichomonads in | | 17 | 15 | 4 | 12 |
| No essential change in numbers of organisms seen in | — | 3 | 4 | 6 | 17 |
| Total cases seen | | 77 | 76 | 39 | 62 |

As regards clinical effects, 47 patients were relieved of their leucorrhoea or pruritus, a similar proportion to those who showed reduction in the number of, or elimination of, the trichomonads. Local reactions were minimal. If no other remedies were available for this distressing condition, no doubt anisomycin would be a useful line of defence, but the method of application is not convenient, nor is the outcome sure, so that patients are not likely to be willing to undergo treatment unless other methods have failed.

PUROMYCIN

Puromycin, known by the trade name of *Stylomycin*, had the unique advantage of not only arresting the growth of trypanosomes but also of being able to kill them. It was prepared from *Streptomyces albo niger* by Porter, Hewitt, Hesseltine, Krupka, Lowery, Wallace, Bohonos, and Williams (1952), who first called the antibiotic achromycin. *Trypanosoma equiperdum* and *Trypanosoma cruzi* were both controlled by its action (Hewitt, Wallace, Gumble, Gill and Williams, 1953, and Pizza, Prager, and Kmerim, 1953). Work on its effect in experimental infections was carried out by Hewitt, Gumble, Wallace, and Williams (1955). Mice were inoculated intraperitoneally with a saline suspension of these organisms and treatment was begun several hours after the inoculation. These workers considered that the mechanism of action of the drug involved an interference with the synthesis of nucleic acid or nucleoprotein. Further experimental work, confined to trypanosomes, was conducted by Trucão, Nogueira, and Franco (1955). These authors found that single doses administered to mice shortly after inoculation failed to prevent development of the infection, but when the doses were repeated daily for 10 to 12 days infection did not develop. It was also noted that the successful use of puromycin seemed to be closely related to the interval of time between inoculation and administration.

Besides trypanosomes, Gumble, Hewitt, Taylor, and Wallace (1956) tested the drug in infections with oxyurids or tapeworms, using mice as the hosts. By feeding the naturally infected animals with 100 to 500 mg of the drug per kg of body weight, a demonstrable reduction in the number of parasites was achieved, compared with controls. This applied to *Aspicularis*, *Syphacia*, and *Hymenolepis*. Puromycin was also found to have anti protozoan activity. Experiments showing its action against toxoplasmosis were carried out by Christen and Thiermann (1953) and Eyles and Coleman (1954). Its amoebicidal effect was demonstrated by Taylor, Sherman, and Bond (1954).

CLINICAL TRIALS

African Sleeping Sickness

Early clinical trials were begun by Trincão, Franco, Nogueira, Pinto, and Muhlpsfordt (1955). Fifteen patients suffering from African sleeping sickness in Portuguese Guinea were selected for trial. *T. gambiense* was isolated from all of them. Puromycin was administered in a dose of 0.25 G by mouth and repeated daily for 7 to 10 days. As far as the early results were concerned, trypanosomes were no longer found in gland juice 24 to 48 hours after treatment began. Six months later 11 of the original patients remained free of infection but 4 had relapsed, all of these had had changes in the cerebrospinal fluid. There were some unpleasant consequences of therapy: occasional mild headache, nausea, vomiting, and sometimes diarrhoea. When the patients had been observed for 17 months Trincão, Franco, Nogueira, Pinto, and Muhlpsfordt (1956) found 10 who had been consistently free from trypanosomes throughout the period, 4 relapses had occurred, and the 15th patient continued to have abnormal findings in the cerebrospinal fluid.

Amoebiasis

Wilmot (1956) tested the effect of puromycin on amoebic dysentery and compared the effect with that of the tetracyclines. Having the African population around Durban, South Africa, where the disease is endemic, to draw on, there was no difficulty in collecting cases. Twenty-one to 51 patients were selected for each treatment. Those receiving puromycin were given 1.5 G daily, whereas those receiving the tetracyclines were given 1 G daily. When assessing the results by sigmoidoscopy and microscopic examination 27 days after treatment began, Wilmot concluded that the tetracyclines were definitely more beneficial than was puromycin. He summarized his results as follows:

| Results of treatment | Type of treatment and number of cases | | | |
|--|---------------------------------------|-----------------|--------------|-----------|
| | Chlortetracycline | Oxytetracycline | Tetracycline | Puromycin |
| Number of cases | 51 | 49 | 35 | 21 |
| Symptom free, ulcers healed, no amoebae | 48 | 45 | 35 | 14 |
| Ulcers not healed, no amoebae found | 2 | | | 2 |
| Open ulceration, and trophozoites present | | | 1 | |
| Ulcers healed, cysts still in stools | | | | 1 |
| Clinical deterioration, removed from trial | | | | 3 |

Another trial by Alvarez and Moreno (1956) in which patients were treated either with puromycin—10 mg per kg body weight per day—or with puromycin and tetracycline—250 mg of each every 4 hours—showed that puromycin could clear the cysts from the stools but it required the addition of tetracycline to enable healing of proctitis and trophic ulcers to take place. Hepatic abscesses with either series had to be drained before healing took place. No toxic effects were noted and no effect in the faeces on other parasites associated with amoebiasis.

Neoplastic disease

Since the mode of action of puromycin and its analogues appeared similar against trypanosomes and mouse adenocarcinoma, it seemed reasonable to try its effect on neoplastic disease in man. Fifty one patients were chosen by Wright, Dolgopel, Logan, Prigot, and Wright (1955). These were all suffering from advanced, disseminated neoplastic disease so that the trial was a severe one. The dose of puromycin given was 0.25 to 0.75 G by mouth daily. Following 21 days treatment there was a slight but temporary regression of the tumour in 15 patients only, but there was no change in the progressive downhill course of the disease. The patients were not only unrelieved but also suffered from nausea, vomiting, or diarrhoea until the treatment ceased.

From the foregoing summary the outstanding change introduced by puromycin would seem to be the effect on trypanosomiasis. For the other conditions mentioned, except perhaps oxyuriasis which has not been tested clinically, either there are other antibiotics which are better therapeutic agents, or the drug has little or no effect.

NYSTATIN

Fungicidin, known by trade names as *Mycomycin*, *Mycostatin*, *Mystechin*, and *Fungistatin*, or, as it was called later, nystatin, was described by Hazen and Brown (1950). It was obtained from *Streptomyces noursei* (Dutcher, Boyack, and Fox 1953) and was found to be both fungistatic and fungicidal against dividing cells *in vitro* (see Table 13). Trials *in vivo* showed that mice infected with strains of candida or histoplasma developed a milder infection with prolongation of life when they were treated parenterally with the antibiotic than when it was omitted from the treatment. The drug had no demonstrable antibacterial action. Its activity was unaffected by horse blood or serum and the toxicity of the crude material to mice and to rabbits (Drouhet, 1955 a) was limited. Furthermore, Hazen, Brown, and Mason (1953) showed that the administration of oxytetracycline to mice infected with *Candida albicans* was highly lethal but if nystatin were given subcutaneously at the time of infection, or within 2 hours (before or after), it exerted a definite protective effect. Tests *in vitro* and protective trials in mice were made by various workers¹. Of the many strains tested, mostly *Candida albicans* inhibition was found to occur at a concentration between 1.56 and 16 Units per ml.

Resistance to the antibiotic was not readily produced. Of 5 strains tested by Stout and Pagano (1956) 1 only developed some resistance by degrees in serial cultures, but even in this case resistance rose only 4 fold and did not rise higher in spite of continued efforts to make it do so.

Administration

When nystatin was recognized as a chemotherapeutic agent an attempt was made to give it by mouth. Even when doses amounting to 12 G daily

¹ Campbell, Hodges and Hill (1953); Drouhet (1955 a); Fisenberg, Weiss and Flippin (1956); Sternberg, Tarbet, Newcomer, Huddleson, Weir, Wright and Egeberg (1953).

(presumably of an impure preparation) were administered by Newcomer, Wright, Sternberg, Graham, Weir, and Egeberg (1956) no sustained or high therapeutic values were obtained in the blood of patients. These workers then attempted to give the antibiotic by intramuscular injection but severe local pain and tenderness together with fever and chills followed. Intravenous administration was therefore adopted and though earlier batches produced

TABLE 13 FUNGISTATIC ACTION OF NYSTATIN
(SERIAL BROTH DILUTION TESTS)

| Species | No of strains | μg per ml inhibiting growth |
|----------------------------|---------------|--|
| <i>Allescheria</i> | 1 | > 100 |
| <i>Alternaria</i> | 1 | 1.56 |
| <i>Aspergillus</i> | | 6.25 |
| <i>Blastomyces</i> | 1 | 1.56 |
| <i>Candida</i> | 270 | 1.56-20 |
| <i>Cephalosporium</i> | 1 | 25 |
| <i>Coccidioides</i> | 2 | 1.56-6.25 |
| <i>Cryptococcus</i> | 3 | 1.56-3.13 |
| <i>Endamoeba</i> | 4 | 125-250 |
| <i>Endomycopsis</i> | | 3.13 |
| <i>Epidermophyton</i> | 1 | 1.56 |
| <i>Fusarium</i> | 1 | 3.13 |
| <i>Geotrichum</i> | 6 | 1.56-12.5 |
| <i>Histoplasma</i> | 1 | 1.56 |
| <i>Hormodendrum</i> | 1 | 3.13 |
| <i>Microsporum</i> | 2 | 3.13-13 |
| <i>Monosporium</i> | 1 | 100 |
| <i>Penicillium</i> | 3 | 3.13-13 |
| <i>Phialophora</i> | 1 | 13 |
| <i>Phoma</i> | 1 | 6.25 |
| <i>Rhizopus</i> | 1 | 3.13 |
| <i>Saccharomyces</i> | 1 | 3.13 |
| <i>Schizosaccharomyces</i> | 1 | 1.56 |
| <i>Sporobolomyces</i> | 1 | 3.13 |
| <i>Sporotrichum</i> | 1 | 13 |
| <i>Trichophyton</i> | 3 | 3.13-6.25 |

Bacteria—no inhibition at 100 μg per ml—*Staph aureus*, *Str haemolyticus*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhosa*, *Shigella dysenteriae*, *Bacterium mucosum capsulatum*, *Bacillus circulans*, *Mycobacterium tuberculosis*

From the data of Drouhet (1955 a), Eisenberg, Weiss, and Flippin (1956), Hazen and Brown (1951), and Stewart (1956)

thrombophlebitis, later ones were less irritating. With an injection of 200,000 Units blood levels ranged between 10 and 18 μg per ml and, when this size of dose was repeated 6 hourly, they rose to 40 to 45 μg per ml—concentrations well over those required to inhibit many fungi. In spite of these findings, however, the oral route of administration, with doses about 5 times those originally given, has been used with apparent good effect in the treatment of moniliasis.

Local administration has been relied on as much as possible. For this purpose nystatin has been incorporated in an ointment, solution, a powder, troche, suppository, pessary, or gel, the amount in each vehicle usually varying between 10,000 and 100,000 Units.

CLINICAL TRIALS

Moniliasis

The most obvious application of nystatin in the clinical field was in the treatment of moniliasis. Sloane (1955) reported the results of treatment by troches containing 3,000 Units, ointment containing 50,000 Units per G, vaginal suppositories each containing 30,000 Units, or a freshly made solution in propylene glycol containing 150,000 Units per ml. Sloane observed prompt responses in the 8 cases of moniliasis of the skin or mucous membranes which he treated, but the condition tended to relapse when the antibiotic was stopped. The common occurrence of thrush enabled Huang, Kendall, Lambert, and High (1956) to treat a series of 20 infants with this condition, 8 of them premature. The appearance of the lesions was typical and the diagnosis was confirmed by the presence of *C. albicans* in scrapings or cultures from the lesions. These infants received local applications of nystatin for 3 to 13 days, by the end of which time the treatment was found to have been effective. A more extensive trial was carried out by Wright, Graham, Newcomer, and Sternberg (1956). These authors selected 96 patients for study, 30 with oral, 17 with vaginal, and 49 with cutaneous moniliasis. Nystatin was applied topically and the results were found to be good in all cases. There were no instances of resistance developing after prolonged or repeated use, and there were, in this series, no sensitivity reactions. Local treatment was also applied to about 300 patients with thrush, vaginitis, or cutaneous moniliasis by Jennison and Llewelyn-Jones (1957), Pace and Schantz (1956), Stovall, Page, Witte, Reuss, and Overstreet (1956), and Wright, Graham and Sternberg (1957). One hundred thousand Units applied daily or on alternate days as ointment, solution, suppository, or pessary brought about alleviation of symptoms in about 1 week in the majority of cases, but *C. albicans* was still found in a number of patients who had clinically recovered. Recurrences were therefore to be expected and did in fact occur. Pace and Schantz (1956) drew attention to the fact that nystatin had only proved effective in cases of vaginitis from which monilia had been isolated. Recurrences were more frequent in patients who were pregnant than in those who were not. When the recurrences were treated again with nystatin, however, a response was still elicited. R. C. V. Robinson (1956) studied the effect of nystatin in infections of the skin and mucous membranes. Sixty-two cases were treated, the most dramatic effect being produced in those with vulvovaginitis. An ointment containing a combination of nystatin 100,000 Units per G with 10 per cent of hydrocortisone brought relief to these patients within 24 to 72 hours.

Treatment of moniliasis by means of oral administration seems to have been productive of more certain results. Stovall *et al.* (1956), who employed both local and oral administration, concluded that of the two methods oral ingestion of undiluted nystatin suspension appeared to give the better results. Their patients, however, were infants and children so that the dose they used—100,000 Units 4 times a day—would be a relatively large one. Moreover, ingestion of so large a dose would leave high concentrations within the mouth to produce a topical effect. The possibility of a systemic effect being produced by ingestion was more feasible in another case. This was also an infant whose course under treatment with nystatin was observed by Beckman and Navarro (1955). The child had a

manifest oral infection on the 10th day after birth, which had not responded to various topical applications nor to any antibacterial drugs administered. The general condition of the patient had deteriorated and there was respiratory distress. Nystatin was administered as a flavoured powder in a dose of 175,000 Units or 1 teaspoonful mixed with an ounce of milk every 6 hours. The oral lesions showed improvement within 48 hours but clinical and radiological improvement of the pulmonary condition was also evident within 2 weeks. The baby was discharged apparently clear of its infection 1 month after admission. Further satisfactory trials were made by Drouhet (1955 *b*) whose patients suffering from generalized or localized lesions received oral doses of 0.1 to 0.2 G per kg of body weight per day for infants and 1.2 to 6 G daily for adults. Four days treatment caused the disappearance of digestive, urinary, and oral disturbances together with the disappearance or diminution of monilia in mouth, blood stools and urine. Weiss, Eisenberg, Sass, Kayser and Flippin (1956) successfully treated 5 patients with vaginitis or pulmonary moniliasis. Nystatin was given in a dose of 4 million Units by mouth daily for nearly 2 months to the patient with pulmonary disease. During this period *C. albicans* disappeared from stool and urine although not from the sputum and radiographs showed much clearing of the condition in the lungs. Of 3 patients of R. C. V. Robinson (1956) with systemic moniliasis all responded to the drug by mouth in doses varying in size according to their age up to 500,000 Units 4 times a day. Further trials were made by Stewart (1956). Twenty patients with monilial stomatitis and pharyngitis were treated, first by local application and later by mouth in 3 to 4 doses daily amounting to 1 to 2 million Units per 24 hours. With both regimes the lesions subsided in a few days and swabs were free of monilia. In 9 out of 12 patients in whom the infection had followed directly on the administration of antibacterial therapy the organism was cleared from the sputum. Stewart also noted that there was no change in the degree of sensitivity *in vitro* of strains of candida re-isolated from patients after treatment had ceased. A temporary effect only was produced in 2 cases of ringworm.

To 5 cases of severe vulvovaginitis due to monilia Sarewitz (1955) administered 500,000 Units by mouth 3 times a day. Rapid clearing of the lesions followed together with relief of itching and soreness. Unfortunately relapse followed discontinuance of treatment.

Coccidiomycosis

Patients suffering from coccidiomycosis treated by Newcomer *et al* (1956) did not respond so well as the patients with moniliasis. These authors described 5 cases, in 3 of which the lesion was in the lungs and in 2 in the meninges. Those suffering from pulmonary lesions did not respond to relatively high doses given daily by mouth, but when the intravenous route was used and 200,000 to 400,000 Units were injected daily, the patients' symptoms improved and they gained weight. It required 4 to 5 months treatment, however, for radiographs to clear sufficiently for the disease to be considered inactive and even after so long a treatment as this sinuses which had healed broke down again during the following year. There was no sign when recurrence took place that the infection would not respond to further treatment, the greatest difficulty being in finding patent veins for the earlier

batches of nystatin had an irritant action on the vein wall. Two patients with meningitis failed to respond to treatment. This was understandable since none of the antibiotic was detected in the cerebrospinal fluid after intravenous administration.

Complications of therapy

Only mild reactions were observed following oral administration of nystatin. Transient nausea was observed by Stewart (1956) after doses of 500,000 Units 3 to 4 times daily for up to 7 days, no ill effects were noted by Sarewitz (1955) even in 1 patient receiving the drug for 3 months. With the earlier preparations for intravenous use, Newcomer *et al.* (1956) observed chills and a rise of temperature as high as 102° F within a few hours of administration. These subsided, however, leaving the patient in better condition after his therapy. Otherwise there have been few reactions which could be attributed unequivocally to nystatin.

Treatment with nystatin combined with other antibiotics

Nystatin and tetracycline

On the assumption that gastro intestinal side effects following antibacterial therapy are due in part to the supervention of resistant infections, moniliasis being amongst these, efforts have been made by various investigators to compare the incidence of these side effects following tetracycline given by itself or together with a fungicidal drug such as nystatin. Stone and Mersheimer (1956) studied 509 patients from this point of view. They noted that these side effects were present in 11.14 per cent of the 260 patients treated with tetracycline and in only 3.16 per cent of those treated with the 2 antibiotics together, and that the differences in the incidence of side reactions were mainly due to those in the alimentary tract except for vaginitis in women which was distinctly more common in those taking tetracycline alone. Stone and Mersheimer (1956) also noted that 6 of the 8 patients treated with tetracycline in whom this complication occurred showed an overgrowth of monilia in their vaginal smears. In neither of the 2 cases of vaginitis in the series treated with nystatin and tetracycline could monilia be demonstrated. On the other hand, in a small series of patients whose reactions were critically assessed by Hewitt, Finegold, and Sutter (1956), taking into account the duration of treatment, little difference was noted in the incidence of reactions in two groups: one containing 77 patients treated with 0.25 G to 0.5 G of tetracycline 6 hourly and another containing 96 patients treated with the same dose of the latter drug together with 250,000 Units of nystatin. The incidence of side reactions was 71 and 78 per cent respectively. The main differences were again found in reactions in the lower gastro intestinal canal, the study being largely confined to men.

Hewitt *et al.* (1956) did, however, observe that there was a significant decrease in the number of yeasts in the faeces following administration of tetracycline and nystatin together compared with patients treated with tetracycline alone. Childs (1956) also ascribed the presence of diarrhoea to a heavy growth of *C. albicans* in the stools. Fifty young males over 12 years of age suffering from pneumonia were divided into two groups. Each alternate case received either 0.25 G of tetracycline 4 to 6 hourly for 5 days or

else the same dose of tetracycline together with 50,000 Units of nystatin 8 hourly (1 Unit = the amount of nystatin present in 1 ml of broth which completely inhibits a strain of *C. albicans*) Side effects in the form of diarrhoea were seen in 4 of the boys taking tetracycline alone, and from the stools of 3 of these heavy growths of *C. albicans* were obtained. No patients, however, developed either diarrhoea or a heavy growth of *C. albicans* in the group receiving combined treatment. Possibly these side effects, as Kligman (1956) would have us believe, have little to do with monilia, their overgrowth being merely coincidental with the symptoms.

In support of Kligman's conclusion are the findings reported a year later. With the same 2 kinds of treatment Lepper and Pearson (1957) found the aerobic bacterial faecal flora after 5 days little altered but candida had increased in distinctly fewer patients receiving nystatin with tetracycline than in those receiving tetracycline alone. As regards side effects there were 4 patients who had diarrhoea after the combination but 2 only after tetracycline alone, nor did the diarrhoea seem to be associated with any particular microbe in the faeces. In the same way neither Metzger, Steigmann, Jenkins, Pamukcu, and Kaminski (1957) or Range, Oriatti, and Engling (1957) could relate the presence or absence of side reactions with the effect of nystatin on candida in the stool.

Pneumonia Twenty five patients suffering from acute pneumonia were treated with tetracycline and nystatin by Gimble, Shea, and Katz (1956). Pneumococci were isolated from 70 per cent of those having positive cultures and in 2 a bacteraemia was demonstrated. These patients were treated with 0.5 G of tetracycline and nystatin. Sixteen of them made an excellent recovery with marked subjective and objective improvement within 2 days, while 4 in whom there was a suspected underlying bronchiectasis or obstructive emphysema improved over a longer time. Even those with pyelonephritis responded well, 2 taking longer to recover than the others. A patient with a streptococcal pneumonia died, as also did another with tuberculosis. Kayser, Weiss, Eiseuherg, and Flippin (1957) also studied the effect of the 2 antibiotics on a series of 26 patients with bacterial pneumonia (pneumococci were isolated from the sputum of each). From treatment with 500 mg tetracycline hydrochloride together with 500,000 Units nystatin by mouth 6 hourly for 7 to 10 days the results were considered to differ in no essential way from those obtained in these workers' previous experience with tetracycline alone. Fungous infections were sometimes seen in debilitated individuals such as those suffering from prematurity, leukaemia, diabetes mellitus, or from treatment with cortisone and related steroids, and the administration of nystatin to such people should have no unfortunate effects. Other conditions treated similarly by Gimble *et al* (1956), such as pyelonephritis, thrombophlebitis, cellulitis, lung abscess, pelvic abscess with septicaemia, bacillary dysentery, and pharyngitis with tonsillitis, bore out this conclusion.

Nystatin, neomycin, and polymyxin

These antibiotics were used in 24 cases by Spaulding, Rao, Tyson, Zubrzycki, and Harris (1956) to test their effect in reducing the bacterial flora of the faeces. Without nystatin, the other 2 antibiotics were able to reduce the bacterial flora considerably, but had no effect on yeasts and fungi which

grew readily in the specimens from 23 of the 24 cases. When, however, nystatin was added to the treatment so that each patient received 4.5 G neomycin, 200 mg polymyxin B, and 3 million Units of nystatin, divided into 3 doses daily following castor oil and an enema, yeasts and fungi disappeared within the first 5 days of medication in all of the 16 cases studied. When nystatin was used with the other 2 drugs, sterility or near sterility of the gut contents was reached in 7 patients, thus preparing them for intestinal surgery. Later work with neomycin and nystatin (Spaulding, Tyson, Harris, Jacobs, Wildrich, and Johnson, 1957) demonstrated that equally good results were obtained without the addition of polymyxin B. No patients' stools, however, maintained sterility for more than 24 hours.

ACTIDIONE

Actidione, like nystatin, was produced from *Streptomyces noursei* (Hazen and Brown, 1950). An antibiotic with similar properties had also been prepared from *Streptomyces griseus* by Leach, Ford, and Whiffen (1947). The chemical structure of the latter was investigated and identified by Kornfeld, Jones, and Parke (1949). The drug appeared to have a unique glutarimide ring in its structure, and was eventually considered to be identical with the actidione produced from nystatin (Brown and Hazen, 1956). Its antimicrobial activity was confined to yeasts and fungi. Whiffen (1948) reported that it inhibited a number of yeasts when incubated with them at 30° C for 18 hours, at 0.17 µg per ml, but certain species of torula required as much as 25 or even more than 1,000 µg per ml. Some fungi, such as *Cryptococcus neoformans*, were inhibited by 0.24 µg per ml, but others, including *Candida albicans* and *Nocardia asteroides*, required more than 1,000 µg per ml as also did various strains of trichophyton. Actidione is a crystalline substance soluble in water, the lower aliphatic esters, acetone, and chloroform and fairly stable to heat, acid, and alkali.

When given intraperitoneally to dogs, rats or guinea pigs, actidione was detected in the plasma within 30 minutes and persisted for nearly 1½ hours.

Its toxicity when administered to dogs was sufficient to produce vomiting within a few minutes of the intravenous administration of 1 mg per kg of body weight. In man, intravenous administration of a sufficiently large dose produced nausea (Goth and Robinson 1949).

Only one clinical trial has come to the author's notice and that was in a patient described by Wilson and Duryea (1951). This patient began to lose weight in October 1948 but the diagnosis of torula meningitis was not made before July 1949, when a *Cryptococcus neoformans* was isolated. This organism was inhibited by relatively high concentrations of sulphadiazine, chlortetracycline, and penicillin so that the meningitis was first treated by these drugs, but without success. Actidione was administered a month later, both intramuscularly and in a dose of 20 mg intrathecally each day for 15 days. After this, 40 mg was injected intravenously each day. The cerebrospinal fluid became sterile within 2 days of intrathecal treatment, but there were marked signs of cerebral irritation after this route of administration had been used for 15 days. Unfortunately, after a further 18 days of intravenous administra-

tion the torula again appeared in the cerebrospinal fluid. Nevertheless, after observation during 3 further courses of intravenous therapy, the patient was discharged free from symptoms. The outcome in this case was contrasted with that in another in whom the same organism was identified in the cerebrospinal fluid 6 months later. Actidione was not at that time available and routine treatment was adopted. The patient eventually died after 4 months. At necropsy, many cryptococci were found in the cerebrospinal fluid and cerebrum.

THE AMPHOTERICINS

Antifungal agents derived from a streptomycete obtained from soil from the region of the Orinoco River, Venezuela were recovered by Gold, Stout, Pagano, and Donovan (1956) and Vandepotte, Wachtel, and Stiller (1956). There were two of these agents, named by the above authors amphotericin A and B. Both are insoluble in water but soluble in aqueous alcohols, and amphoteric. Amphotericin B was found to be several times more active than A against yeasts and yeast like fungi, but A inhibited a greater number of species of fungi. Neither has any activity against bacteria. Unfortunately the extreme insolubility of the amphotericins is a handicap in clinical application for, though they can be converted into water soluble salts, B rapidly loses its activity in the process. Each was crystallized and, when dry and stored at moderate temperature away from light or air, was stable for long periods.

Antifungal activity

When incubated at 30° C or 37° C for 4 days with various fungi, the amphotericins demonstrated their inhibitory activity against the following organisms:

| | |
|---|----------------------------------|
| <i>Candida albicans</i> (various strains) | <i>Cladosporium</i> (2 strains)* |
| <i>Rhodotorula</i> (2 strains) | <i>Fonsecaea</i> (2 strains)* |
| <i>Saccharomyces cerevisiae</i> | <i>Phialophora verrucosa</i> * |
| <i>Sporotrichum schenckii</i> * | <i>Geotrichum</i> sp. |
| <i>Microsporium</i> (3 strains)* | <i>Nocardia</i> † (4 strains) |
| <i>Trichophyton</i> (5 strains) | <i>Aspergillus fumigatus</i> * |
| <i>Monosporium apiospermum</i> * | <i>Fusarium bulbigenum</i> |
| <i>Cryptococcus neoformans</i> | <i>Blastomyces brasiliensis</i> |
| <i>Epidermophyton floccosum</i> | <i>Histoplasma capsulatum</i> |
| <i>Cephalosporium recifense</i> * | |

Amphotericin A resembled nystatin in the type of organism it inhibited and the concentrations required for inhibition. Amphotericin B was more active than A against *Candida albicans*, *C. neoformans*, *B. dermatitidis*, *Blastomyces brasiliensis*, *S. schenckii*, and *H. capsulatum*.

* Much more sensitive to amphotericin A than to amphotericin B.

† Relatively resistant to both amphotericins, requiring > 25 to > 50 µg per ml for inhibition.

Trials in experimental animals such as mice, guinea pigs, and hamsters showed that both antibiotics, when given subcutaneously, could protect the animals against fungal infections such as those due to *C albicans*, *H capsulatum*, *C neoformans*, and *T mentagrophytes*. Amphotericin B showed no evidence of toxicity when administered daily to mice via the peritoneal cavity for 50 up to 71 days (Sternberg, Wright, and Oura, 1956). Baum, Rohel, and Schwarz (1957), using antibiotics alone and with another chemotherapeutic agent, concluded that a combination such as amphotericin B or nystatin, together with sulphadiazine, gave better results than those from a single agent. Because of their poor solubility and tendency to remain in the subcutaneous tissues where they were injected, thus limiting absorption, these antibiotics were not thought to have produced the best results possible with them. On the other hand Loura, Feder, and Emmons (1957) found good absorption followed oral administration or intraperitoneal injection in mice of up to 150 mg per kg per day and were satisfied that amphotericin B was highly effective in its protective action against experimental histoplasmosis and cryptococcosis. Because of its poor solubility a mixture with sodium desoxycholate was prepared by Bartner, Zinnes, Moe, and Kulesza (see *Lancet*, 1957, ii 888) which, when reconstituted with 5 per cent dextrose in water, formed a colloidal dispersion rather than a solution. No essential difference was found in the toxicity to rabbits and dogs between this and the original suspension in water.

CLINICAL TRIALS

Most commonly the amphotericins would be required to treat cutaneous candidiasis or thrush. Kozinn, Taschdjian, Dragutsky, and Minsky (1957) therefore attacked the disease in 37 newly born infants and young children. Treatment was applied by local injection with ointments containing 2 per cent of amphotericin B or of nystatin. In early cases, i.e. of not more than 3 days' duration either ointment was uniformly successful, clearing up the lesion in approximately a week. When the candidiasis was more advanced success was not so great and longer treatment was required. In these cases nystatin produced better results than amphotericin but the authors point out that an underlying dermatosis, if present, requires treatment as well.

The effect of amphotericin B was also tried in the yeast flora of the gastrointestinal canal. The 24 male patients on whom the trial was made by Halde, Wright, Pollard, Newcomer, and Sternberg (1957) were suffering from skin complaints and had no known abnormalities of their alimentary canals. Serial yeast counts of their stools before and after amphotericin B, given as 50 to 300 mg 4 times a day in capsules by mouth, showed that these decreased by 73 to 100 per cent in the great majority of instances. When amphotericin B was administered with tetracycline to counteract the effect of the latter, the yeast counts were still reduced to a greater degree when 800 mg a day were administered than after a lower dose of 200 mg a day. When considering the antibiotics for routine treatment with tetracycline it is as well to bear in mind that in certain strains of *Candida* (not *Candida albicans*) resistance to a high degree has been induced *in vitro* and this, in the case of amphotericin B, exhibited cross resistance with nystatin (*Lancet*, 1957, ii 888).

Amphotericin B was also used for systemic fungal diseases¹ In 1 series of 14 patients where all the patients had a poor ultimate prognosis and had failed to respond to other treatment, the results, whether from oral or intravenous administration, were not particularly encouraging Only 1 case with a cryptococcus infection of his bone and soft tissues recovered Three patients, though showing amelioration of their symptoms, did not recover and others did not appear to be affected by the treatment Of those treated intravenously, 2 showed definite clinical improvement, gaining weight and in one instance feeling well enough to return to work 4 months after his treatment, with wounds healed and drainage from his abscess having ceased A much more optimistic view of the possibilities of therapy with amphotericin B was given in a second series of 6 patients with cryptococcal meningitis Intravenous administration of infusions containing 50 to 100 mg daily or twice a day resulted in 5 patients surviving and 1 death only The survivors were restored to normal activity in 3 cases, a 4th was asymptomatic except for occasional headaches, and the condition of the 5th survivor remained stable after having slowly deteriorated over the previous 10 years

In summing up these remarks about the amphotericins it is of interest to quote Furcolow (1937), who described the results of the treatment of cryptococcosis (torulosis) and blastomycosis as encouraging

THE ACTINOMYCINS

Actinomycin or 'sanamycin' was found by Waksman and Woodruff (1942) as a product of the culture of a species of actinomyces Two crystalline fractions were obtained and called actinomycin A and B In 1949 Brockmann and Grubhofer also isolated an actinomycin, 'Actinomycin C', from *Streptomyces chrysomallus* and found that, in addition to its other properties, this drug also inhibited cellular activity in the Ehrlich carcinoma of the mouse Experiments were extended by Hackmann (1952) to carcinomatous growths in rats Hackmann (1953) observed that the antibiotic had a particular static effect on the cells of the lymphatic system Later a number of different actinomycins were recognized (Gregory, Vining, and Waksman, 1955, Manaker, Gregory, Vining and Waksman, 1955) The original material was found to be strongly bacteriostatic against both Gram positive and Gram negative organisms and also to be fungistatic When incubation was carried out for 4 days at 28° C a concentration of 0.1 mg per ml was sufficient to prevent the growth of species of *Rhizopus*, *Trichoderma*, *Penicillium*, *Humicola*, *Fusarium*, *Aspergillus niger* and *Candida* and of much of the bacterial and fungal population of the soil

Clinical trials were begun by Schulte (1952) on patients who had tumours of the reticulo endothelial system, but the results were indefinite Some patients showed improvement with disappearance of their tumours, and others a reduction in the radiation dose required to control them, but these

¹ These cases are referred to in the Annotation in the *Lancet* (1957, ii 888) which describes the results of reports made at the Fifth Annual Symposium on Antibiotics, October 1957

changes did not occur consistently. Later trials (Schulte, 1954), in which intravenous injections of 100 to 200 μg were given morning and evening until 5,000 to 10,000 μg had been administered, encouraged Schulte to believe that actinomycin C was of therapeutic value either alone or in conjunction with radiotherapy, urethane, nitrogen mustard, or cortisone. Ten of 17 previously untreated patients with Hodgkin's disease were free from signs and symptoms after one course and remained so for periods up to 18 months. Some who were of working age were able to return to their employment. The best effects in all groups of patients studied were found in patients with only superficial lymph node involvement, and no pyrexia, or with an increase in erythrocyte sedimentation rate and no gross blood changes. The toxic effects of therapy appeared to be mild. After treating 100 cases Schulte had noted no detrimental changes in liver, kidneys, or haemopoietic system, but it is as well to bear in mind that experimental work with guinea pigs carried out by Businco (1955) showed that the drug did not prevent anaphylactic shock in sensitized animals. Moreover, in 1955, Janbon, Bertrand, and Carli reported some fatalities from agranulocytosis following treatment with relatively high doses of 1,000 μg daily for about 2 weeks. A large series of cases, 118 in all, treated by French workers was reported in 1954 by Bertrand, Fontaine, Mallarme, Schneider, and Dchray. These were mainly cases of Hodgkin's disease and the reports were uniformly unfavourable, except for that of Ravina and Pestel (1954). The latter workers treated 13 patients at the Hopital Beaujou, Clichy. Bearing in mind that urinary excretion of the drug was rapid, treatment by intravenous drip was adopted and up to 800 μg were given over 3 to 4 hours, thereby ensuring a more or less constant level of the antibiotic in the blood during that time. The other French workers gave daily injections, thus achieving only transiently high concentrations. In all of 4 cases of Hodgkin's disease treated by Ravina and Pestel, remissions took place, and remissions occurred also in 4 others with carcinoma of the liver, lymphosarcoma, lymphoid follicular reticulosis, or malignant ascites. Other forms of cancer in a further 5 cases, however, did not respond. The French workers noted that unpleasant side effects included anorexia, nausea, abdominal pain, diarrhoea, stomatitis, alopecia, and jaundice. Similar side effects of therapy were noted by Croizat and Lacoste (1955) whose 44 patients underwent treatment for various malignant adenopathies. Unfortunately, with daily intravenous injections of 200 to 400 μg according to age and sex, these workers saw no definite benefit and considered the results of radiotherapy or nitrogen mustard much superior. Other workers have had little more success. In Hodgkin's disease, Korst and Meyer (1955) saw no effect after administering 25 μg of the pure crystalline actinomycin D daily by intravenous injection for 9 to 30 days. Trounce, Wayte, and Rohson (1955) gave a total dose of 7,000 to 10,000 μg with only slight and transient subsidence of fever and reduction in the size of lymph nodes in 2 out of 4 patients (2 of these patients also showed a distinct thrombocytopenia). Magnus and Zeitler (1955) could not come to a decision as to whether the action of the drug was beneficial either in this disease or myeloid leukaemia. Schmidt, Loosen, and Hemen (1955) claimed success with patients suffering not only from Hodgkin's disease, but also various malignant tumours. They stated that it was essential to maintain prolonged treatment, administering 50 to 400 μg intravenously each day for weeks or months so

that the total dose might reach 1,675 to 19,000 μg in Hodgkin's disease and as much as 45 000 μg in patients with malignancies. With this long treatment Schmidt *et al.* (1955) claimed that all of their 8 patients with Hodgkin's disease, followed for 5 to 29 months, had improved in their general condition and their anaemia. In 4 the lymph nodes and metastases had regressed in size. In patients with malignant tumours followed for 3 to 37 months, subjective improvement had been experienced in 10 out of 12 and metastases had demonstrably regressed in 2. After operation on 1 for hypernephroma, the clinical recovery was complete and the metastases in the lungs had diminished in size. These more or less satisfactory results were also obtained at a later date by Ravin, Pestel Eloy, and Thülen (1956 b). These workers described beneficial effects produced by actinomycin C in malignant blood disorders, a splenic form of Brill-Symmers disease, cancer of the uterus with pulmonary metastases and ovarian cysts which were treated by direct instillation.

With these somewhat contradictory statements on the effect of actinomycin, judgement about its place in the treatment of neoplastic disease must for the present be withheld. Since the antibiotic has also an antibacterial action, confirmed as late as 1956 by Foley, it is possible that some of the beneficial effects observed may have been due to subsidence of inflammatory reaction around malignant foci. Nevertheless the results from prolonged treatment given by Schmidt *et al.* (1955) give ground for hopes that when this is adopted, especially in early cases, greater benefits will accrue.

SARKOMYCIN

In 1953 Umezawa, Takeuchi, Nitta, Yamamoto, and Yamaoka (1953) reported the preparation of an antibiotic from a streptomyces similar to *S. erythrochromogenes*. The antibiotic was acidic in nature and weakly antibacterial against *Staph. aureus*. Although it was not crystallized when reported on by Hooper, Cheney, Cron, Fardig, Johnson, Johnson, Palermati, Schmitz, and Wheatley (1955), its toxicity was low, the LD_{50} for mice being 800 to 1,600 mg per kg of weight by intravenous injection, 400 to 800 mg per kg by subcutaneous injection and 4,800–6 400 mg per kg by oral administration. In experimental work in mice, daily administration of 1 mg intravenously, 2.5 mg intraperitoneally, or 5 mg by mouth prevented the development of Ehrlich sarcoma.

Clinical trials were carried out by Ishiyama (1954) on 78 inoperable cases of malignant tumours in Kanto Teishin Hospital, Tokyo. Ishiyama considered that clinical improvement followed treatment in 26 patients and this was accompanied by histological and radiological confirmation. There were few side-effects from the treatment and they were considered negligible. Further work has not yet come to the authors' notice.

AMICETIN

Produced from *Streptomyces tinaceus drappus* and *Streptomyces fasciculatus* by De Boer, Caron, and Hinman (1953), amicetin was first found to be active in low concentrations against *Mycobacterium tuberculosis* and *Staph aureus*. The properties of this antibiotic were described after crystallization by Flynn, Hinman, Caron, and Woolf (1953). It is a colourless substance, insoluble in water and most organic solvents. It is amphoteric and dissolves in dilute acids or bases but is highly unstable in alkaline solution. Although it showed activity against acid fast mycobacteria and *Staph aureus*, because of its chemical constituents its main interest lay in the favourable effect it produced on the survival time of mice with experimentally induced leukaemia (Burchenal, Yuceoglu, Dagg, and Stock, 1954).

Tan and Burchenal (1956) made a clinical trial with this antibiotic on 11 patients with acute leukaemia and 4 with other neoplastic disease. They injected the drug intravenously, gradually increasing the dose till it reached 2 G or 100 mg per kg of body weight, infused in 1,000 ml of 5 per cent glucose in water over 24 hours, or up to 12 G daily by mouth (higher doses than those mentioned were not tolerated). Unfortunately there was no indication that amicetin had any therapeutic value in acute leukaemia except possibly in 1 child to whom amicetin was given by continuous intravenous drip for 3 weeks and by mouth for another 2. After the 4th week of therapy the child's peripheral leucocyte count was unchanged but the percentage of stem cells in his bone marrow had fallen from 90 to 15 and was still not more than 30 when he died during the 5th week of therapy from bronchopneumonia.

OTHER ANTIBIOTICS ACTIVE AGAINST MALIGNANT CELLS

Products derived from a large number of infective agents, with antiblastic activity for example the polysaccharides of *Chromobacterium prodigiosum* and other Gram negative bacteria, diphtheria toxin, *Bacillus subtilis*, bacteriophage, viruses, fungi, malaria parasites, entamoeba, leishmania, and *Trypanosoma cruzi*, have been tested by Klueva and Roskin (1957). Some of these extracts notably that from *Trypanosoma cruzi*, have reached the stage of clinical trials and from these Klueva and Roskin have concluded that they have great potentialities in the treatment and control of malignant tumours, regression or disappearance following treatment having been observed in some tumours of the lips, vocal cords, throat, breast, oesophagus, intestine, and skin.

Such findings await confirmation in other countries.

CONCLUSION

At the present time, though there are indications that antibiotic substances may influence neoplastic disease, the grounds for considering that any agent so far discovered is capable of rendering the rapidly multiplying cells of cancer inactive are too insecure to offer any hope from them of a curative effect.

CHAPTER 7

THE CHOICE OF AN ANTIBIOTIC

GENERAL CONSIDERATIONS

Sensitivity tests

BEFORE deciding to treat with an antibiotic certain considerations need to be taken into account. Amongst these, the sensitivity of the responsible organism ranks in the forefront. Other points to be taken into consideration are the increasing incidence of resistant bacteria, the possibility of complications arising from each antibiotic, and the manner of dealing with them. There is also the question as to whether certain agents such as the corticosteroids or gamma globulin may aid an antibiotic in speeding the patient's recovery. These will be dealt with in the following pages.

Logically, the antibiotic to be chosen for any infective process should be that to which the responsible organism is most susceptible. Even when the organism can be isolated, however, there are some objections to this method of choice. To begin with, the method of testing for susceptibility has in it many sources of error which make it questionable whether this test always represents a replica of the susceptibility of the organism *in vivo*. For instance, the constituents of the culture medium may influence the activity of the drug in question, the pH of the medium, the length of incubation, the solubility and diffusibility of the antibiotic in the medium, and the size of the bacterial inoculum. When tests are carried out in solid media these sources of error may be increased. Moreover, arbitrary concentrations are often used in disks or agar dishes, and these may be much above those required to inhibit an organism in liquid media. The diffusibility of the antibiotic through the agar, the concentration of the drug in the tablets or disks, and different rates of increase in the diameter of the zones of inhibition, must be taken into account. An example of the great variation obtained by different methods of testing the same strain of organism was given by Jackson and Finland (1951). These authors tested 7 different antibiotics and 6 different strains of bacteria by 5 methods in common use. With the same strain the variations in susceptibility to the antibiotic were found to vary with the method used and were always at least 2 fold and sometimes as much as 250 fold. Moreover, the correlation between different sensitivity tests was not always the same for each antibiotic. With penicillin clear end points were obtained with most of the methods used except the turbidimetric method, in which discrepancies amounting to a 16 to 64 fold variation were sometimes found. With streptomycin the variation between different methods of testing was not great, providing the inoculum was not heavy. With chlortetracycline the length of incubation was a critical factor, since prolonged incubation allowed the antibiotic to deteriorate and organisms which had been inhibited but not killed to grow out. Correlation between different tests was good with chloramphenicol, except in the case of the

medicated disk method, and when the staphylococcus was the test organism Oxytetracycline, on the other hand, showed variable results. Neomycin, when tested against *Salmonella typhi*, gave relatively consistent results except with the turbidimetric method. Moreover, there is not always certainty that commercially prepared medicated disks contain the concentration claimed for them. Among 92 lots of commercial paper type disks tested by Greenberg, Fitzpatrick, and Branch (1957) 62 had less than their labelled potency. Although it is to be hoped that such errors have been corrected, the method of testing by disk must be regarded only as approximate. Apart from these technical sources of error, there is always a possibility that an organism may be quite sensitive *in vitro* at the beginning of treatment but later acquire a resistance which puts it beyond the reach of therapeutic control. An example of the confusion which may result from direct interpretation of sensitivity tests as a guide to therapeutic control was given by Forbes (1953). Forbes tested the minimal concentration of streptomycin, chloramphenicol, chlortetracycline, and oxytetracycline which would inhibit 17 different strains of *Shigella sonnei*. The absence of turbidity or bacterial deposit was the criterion of complete inhibition. When the tests were read after 12 hours incubation, *Sh. sonnei* appeared to be most sensitive to chlortetracycline. When, however, they were read after 24 hours incubation, chlortetracycline showed less activity than tetracycline. Chloramphenicol and streptomycin appeared to be least effective. According to the tests used for estimating acquired resistance streptomycin appeared to be the least suitable drug. However, when known numbers of the shigellae were incubated for 24 hours at a pH of 7.4 in wet faeces to which the drug was added at a concentration of 10 μ g per G, the only antibiotic which killed all the organisms in this time was streptomycin. This last test proved to be the most reliable, for when streptomycin, in doses of 0.5 G by mouth twice daily, was administered to 16 cases of acute dysentery and to 1 case of a carrier for 2 to 3 days clinical and bacteriological cure was achieved in all except 1, in which cure did not occur until the 6th day after treatment, and no relapses were seen in the subsequent 6 months. Another example was given by Smith and Galloway (1953), who attempted to treat diarrhoea of infants with the antibiotic indicated by a tube dilution test. The predominant organisms isolated from the faeces in these cases were several different strains of *E. coli*. According to these tube dilution tests oxytetracycline had the most powerful inhibitory action on these organisms, yet in patients given this antibiotic the stools were not cleared of organisms any more quickly than when no antibiotic was employed. In the control group of 33 infants the stools became free of organisms in an average of 16 days.

In spite of the many disadvantages inherent in sensitivity tests in general there is something to be said for the adoption of one single test by routine laboratories. This at least rules out the many details of procedure in which variations may occur and allows a correlation to be made between the sensitivity test and the clinical progress of the disease in patients served by a particular laboratory. In choosing a test for routine use, however, certain rules should be observed. These have been set down by Finland (1953 a) as follows:

The medium must support early and good growth of both the infecting and the test organism.

The medium must not interfere with the action of the antibiotic

The period of incubation should be long enough to allow full or nearly full growth in the control medium

No significant deterioration of the antibiotic should occur during the period of incubation

Even so, it is still sometimes necessary to limit the tests to those strains of bacteria which have significant variations in susceptibility. One of the quickest tests is the disk method introduced by Bondi, Spaulding, Smith, and Dietz (1947). These disks contain suitable concentrations of various antibiotics and are placed on the surface of blood agar plates immediately after these have been inoculated with the material from the lesion to be investigated. The organisms are considered to be sensitive when an inhibitory zone of measurable width surrounds the disk. This method, with certain modifications, was used by Lind and Swanton (1952) and Land (1953). Land found a good correlation between the sensitivity of the organism as shown by this test and clinical cure in 50 cases infected with common pathogens. The correlation was not so good in the case of staphylococci or proteus organisms. A similar method described by Broom, Martincau, and Young (1953) was found to correlate well with the clinical course of the disease in most of 69 cases of meningitis due to various organisms. Tunevall and Ericsson (1954) found that the correlation between clinical results and susceptibility as shown by this same method was particularly good with streptomycin, penicillin, and the tetracyclines, but very much less so with chloramphenicol. A more cautious estimate was made by Weil and Harris (1953). These authors found no instance in which the clinical experience was found to be contradictory to the sensitivity tests.

Other methods which depend on the diffusion of the drug into solid media on which a direct culture from the lesion has been made, have been described and recommended by Knox (1954). These methods, according to Knox, are good enough to give guidance as to whether or not the organism in question is sensitive to the drug but they are not accurate enough to allow a quantitative report of the sensitivity to be given with any precision. For quantitative estimations a serial tube dilution method, used with the precautions suggested by Waisbren, Carr, and Dunnette (1951), seems to be the most satisfactory, particularly for cases of bacterial endocarditis. With all the foregoing liabilities to error in sensitivity tests it is satisfactory to find that a study of 118 samples from patients carried out by Rodger, Branch, Power, Starkey, Gregory, Murray, and Harrop (1956) with carefully checked sensitivity tests led them to the conclusion that the results of therapy correlated in a high percentage of cases with the sensitivity tests.

Acquisition of resistance to antibiotics

Increasing resistance of a micro organism to an antibiotic seems to have been observed first in the clinic in cases of bacterial endocarditis. In these cases, where treatment was prolonged and the risk of superadded infection is small, it is possible to assume that one is dealing with the same strain of organism from start to finish of therapy. Astler and Morgan (1950) gave examples of 2 cases of bacterial endocarditis due to *Strept faecalis* or *Staph aureus*. The case due to the streptococcus was treated with sulphonamides,

penicillin, and streptomycin. In this case the streptococcus was at first inhibited by 0.4 to 4 Units per ml of penicillin, but after a recurrence of the infection and a pulmonary infarction, the organism was not inhibited by 5 Units per ml of penicillin. This result might have been within the limits of experimental error. The 2nd case, due to a penicillin resistant *Staph aureus*, was treated with chloramphenicol for 2 weeks, then penicillin and streptomycin, and eventually chlortetracycline for 32 days. The staphylococcus was originally inhibited by 12.5 μ g of chloramphenicol per ml but after 2 weeks' treatment more than 200 μ g per ml were required for inhibition. When chlortetracycline was administered the susceptibility of the organism to it steadily diminished from an initial inhibitory level of 0.2 μ g to 26 μ g per ml during 24 days' treatment.

Pansy, Khan, Pagano, and Donovick (1950) found that they could not only make strains of *E coli* and *Staph aureus* resistant to chlortetracycline and chloramphenicol *in vitro*, but that those made resistant to chlortetracycline were also resistant to its analogue, oxytetracycline. Similar cross resistance was observed between penicillin and penicillin X by Eisman, Marsh, and Mayer (1946) and Dowling, Hirsh, and O Neil (1946) and between streptomycin and its related compounds by Rake, McKee, Pansy, and Donovick (1947), and by others. Gezon and Fasan (1951) induced a 12- to 3,500 fold increase in resistance in 7 strains of group A β haemolytic streptococci after 47 serial plate transfers in increasing concentrations of streptomycin, neomycin, chloramphenicol, chlortetracycline, or oxytetracycline. Gocks and Finland (1951) made repeated subcultures of 14 aerobic pathogens on solid media and not only induced resistance to chlortetracycline, oxytetracycline, chloramphenicol, and neomycin, but found also that cross resistance had been induced between various analogues. Between chloramphenicol and the tetracyclines cross resistance was variable. Induced resistance to streptomycin was the most constant finding, but there was no cross resistance between streptomycin and other antibiotics, including neomycin, although the reverse did not hold true. Wright, Purcell, Wilcox, Broderick, and Finland (1953 and 1954) found that resistance could be induced in staphylococci by repeated subcultures in increasing concentrations of penicillin, streptomycin, erythromycin, and the tetracyclines, and even in certain streptococci. Resistance could be induced in strains of *E coli* to streptomycin, chloramphenicol, oxytetracycline, or polymyxin. These few examples show that there is ample evidence that resistance to antibiotics can be induced in almost any organism *in vitro*. As far as the development of resistant organisms in the clinic is concerned, however, the picture is not so clear. Apart from streptococci isolated from cases of bacterial endocarditis, resistance in bacteria other than staphylococci has not been so commonly reported. Dowling (1954) stated that resistance in β haemolytic streptococci and pneumococci, although demonstrated *in vitro* had not been found in patients. The explanation given by Dowling, Lepper, and Jackson (1955) for the fact that certain infections seldom, if ever, become resistant, is that the response to treatment is prompt and the patient is rid of the infection quickly. This does appear to be the case in infections due to pneumococcus, meningococcus, β haemolytic streptococcus, gonococcus, shigellae, and *Haemophilus influenzae*. When the response is slow an opportunity is afforded for resistance to develop, for example, in infections due to α and γ streptococci, coliforms, proteus,

pseudomonas, staphylococci, and *Myco tuberculosis* Dowling *et al* (1955) explained the relapses seen in infections which seldom become resistant, such as brucellosis, typhoid fever, and rickettsial disease, as due possibly to the fact that some of the organisms are intracellular and therefore beyond the reach of most antibiotics. Whether or not this is so, an increase in the percentage of strains resistant to antibiotics among pathogens isolated from clinical material has been observed from year to year by more than one observer. For example, Meleney and Johnson (1953 *b*) drew attention to this increase in the Presbyterian Hospital, New York, and Thomson (1952) gave the following figures obtained over 3 monthly periods at the Royal Prince Alfred Hospital, Sydney

| Organism | Percentage of strains found sensitive to | | | | |
|---------------------|--|---------------|--------------------|------------------|------------------|
| | Dates isolated | Strepto mycin | Chlortetra cycline | Chloram phenicol | Oxytetra cycline |
| <i>E coli</i> | 1 50-12 50 | 51-53 | 63-49 | 80-72 | 76-67 |
| | 1 51-3 51 | | 32 | 58 | 62 |
| <i>Proteus sp</i> | 1 50-12 50 | 37-74 | 7-8 | 63-7 | 15-2 |
| | 1 51-3 51 | 67-5 | 12-5 | 63-1 | 13-1 |
| <i>Ps pyocyanea</i> | 1 50-12 50 | 50-41 | 6-5 | 17-4 | 47-4 |
| | 1 51-3 51 | 47 | 7-2 | 16-9 | 29-0 |

Similarly, increases in the proportion of strains of α , β , and γ streptococci with diminished susceptibility to penicillin, streptomycin, the tetracyclines, and chloramphenicol were observed yearly between 1949 and 1953 by Kenney, Johnson, and Tatz (1953), and of *E coli*, *A aerogenes*, *Ps aeruginosa*, and the proteus group to each of these antibiotics except penicillin. In comprehensive bacteriological studies of clinical material obtained at their respective hospitals, Jones, Feldman, and Finland (1957), Jones and Finland (1957 *a* and *b*), and Rantz and Rantz (1956) observed the changing sensitivity to antibiotics of various pathogenic bacteria. An increasing proportion of strains during the years 1949 to 1955 were found to be less sensitive to the antibiotics then in use (penicillin, streptomycin, bacitracin, chlortetracycline, and chloramphenicol) than at the beginning of their studies. The bacteria showing this tendency included haemolytic and non haemolytic streptococci, enterococci, *Micrococcus pyogenes* var. *aureus*, *Escherichia coli*, paracolon bacilli, and proteus species. In spite of this tendency, however, penicillin still remained more active against haemolytic streptococci *in vitro* than any other antibiotic tested. Alone among bacteria subjected to these tests pneumococci remained equally sensitive throughout the years of comparison.

Resistant staphylococcal infections

So frequently have resistant strains of staphylococci been isolated from lesions in the clinic that this problem is dealt with here separately. In the early days of the clinical application of penicillin, strains resistant to this antibiotic were rarely encountered. In 1942 Rammelkamp and Maxon drew attention to the existence in clinical material of a few strains only inhibited by higher concentrations than were required by those isolated before therapy began. Plough (1945) also made a similar observation and Kirby (1944), in mentioning staphylococci found resistant to penicillin, described the extraction of a highly potent inactivator from these organisms. Finland (1954) drew attention to the fact that before 1946 almost all strains of staphylococcus

isolated from patients of the Boston City Hospital were inhibited by 0.1 Unit per ml of penicillin. Spink (1954) also pointed out that before the earlier date only an occasional penicillin resistant staphylococcus appeared spontaneously in cultures. By 1952, 25 per cent or more of the strains found in the Boston City Hospital were inhibited only by 1 to 100 Units of penicillin per ml or more, and by 1954 more than 50 per cent were highly resistant (Finland 1955 *b*). Similar increases in the degree of resistance and in the proportion of strains resistant to a given concentration of the drug were observed with chlortetracycline, oxytetracycline, and erythromycin.

D'Agata (1957) described the increasing percentage of strains isolated from patients in hospital and resistant to penicillin, streptomycin, chloramphenicol, chlortetracycline, and oxytetracycline during the years 1954 to 1956. Donieger and Parenteau (1957), surveying the tendency of staphylococci to become resistant to various antibiotics over 3 years, found at the end of their survey of 550 cultures that relatively few were sensitive to penicillin, neomycin and sulfisoxazole, some were still inhibited by the tetracyclines and streptomycin but erythromycin and chloramphenicol were found consistently to inhibit the great majority of staphylococci submitted for tests. A further comparative study on the susceptibility of staphylococci to antibiotics, carried out by Elliott and Hall (1957 *a*), still showed the high incidence of strains resistant to penicillin, streptomycin, and the tetracyclines, but strains resistant to chloramphenicol and erythromycin had already appeared while novobiocin, bacitracin, vancomycin and neomycin still held pride of place amongst antibiotics to which staphylococci were consistently sensitive. These findings, obtained in different laboratories attached to different hospitals, support one another except in the case of neomycin. The susceptibility of the staphylococcus to this antibiotic is not of such practical importance however, as to the others.

Effect of use in hospitals on the incidence of antibiotic resistance

Following this bacteriological survey of the trend of increasing resistance in staphylococci to antibiotics it is of interest to observe the clinical impact of this phenomenon. One of the earliest papers to draw attention to the presence of clinical infections due to penicillin resistant staphylococci was that of Barber and Rozwadowska-Dowzenko (1948). Penicillin resistant organisms were isolated from cases of septicaemia, boils and other superficial lesions, from the lungs, urine, vagina, and umbilicus of newly born infants. All these resistant strains were found to be penicillinase producers. With other widely used antibiotics the incidence of resistant strains rose as the drugs became more commonly administered, but with these other antibiotics, the increase in resistant strains was more rapid. For example, when 100 strains had been isolated at the Mayo Clinic in the first 3 months of 1951, 9 per cent were found to be resistant to 6.2 μg per ml of chlortetracycline, but during the last 3 months of the same year 36 per cent were resistant to this concentration (Needham and Nichols, 1953). Discussing the increasing number of deaths from staphylococcal infection in the Royal Children's Hospital, Melbourne, Taft (1955) refers to the fact that few cases were still responsive to chlortetracycline. In the earlier part of 1953 the percentage of strains sensitive to this drug was 90.6 but, after widespread use of the antibiotic, this fell to 7.4 per cent in the latter half of the same year. Cross

resistance between chlortetracycline and oxytetracycline being so constant, the introduction of oxytetracycline had little effect on the susceptibility of staphylococci. Even with chloramphenicol, to which resistance was relatively infrequently seen, there is some evidence that a similar phenomenon can occur. Kirby and Ahern (1953) found that during a 9 month period when chloramphenicol was used in King County Hospital to a greater extent than either of the tetracyclines, 20 per cent of strains were resistant to the drug, while in a later period when the tetracyclines were more commonly used, only 6 per cent of strains were resistant to chloramphenicol. A similar increase in resistant strains concomitantly with the increased use of chloramphenicol was noted by Koch (1956). The most dramatic figures are those given by Lepper, Moulton, Dowling, Jackson, and Kofman (1953 c) for erythromycin. Before this antibiotic was introduced in the Contagious Diseases Hospital, Chicago, in late 1953, all strains of staphylococcus isolated were inhibited by $0.95 \mu\text{g}$ per ml of erythromycin. One month after the introduction of this drug for routine use, 17 per cent of the staphylococci isolated from members of the hospital staff were inhibited only by $1 \mu\text{g}$ per ml and, during the next 3 months, the increase in resistance was so marked that 75 per cent of strains isolated were inhibited only by $100 \mu\text{g}$ per ml.

Such a universal increase in resistance to antibiotics requires some explanation. It was thought at one time that phage typing of staphylococci might reveal one type which had a greater tendency to become resistant than others. Most of this work was done with penicillin and the majority of strains isolated from different hospitals in England were found to belong to the same group (Barber and Whitehead, 1949). Similar work was carried out by Clarke, Dalglish, and Gillespie (1952), Wallmark (1954), Fusillo and his collaborators (Roerig, Metzger, Fusillo, and Ernst, 1953), Jackson, Dowling, and Lepper (1954), and Knight and Holzer (1954). By the time these reports were made most of the strains were found to be resistant not only to penicillin but also to streptomycin and the tetracyclines, and the majority belonged to group III phage type. However, there was other evidence which did not support the view that a particular phage group was associated with acquired resistance to antibiotics. It was shown experimentally that one type of staphylococcus could be implanted on another in culture, and take its place completely, yet by selective subculture with a suitable antibiotic the original staphylococcus could again be isolated (Fusillo, Carroll, and Ernst, 1955). Metzger, Fusillo, Roerig, and Ernst (1954) went so far as to state that resistant staphylococci were primarily associated with cross infection, one type of staphylococcus replacing another. These authors used phage typing as a means simply of recognizing when one strain had replaced another (Fusillo, Roerig, Metzger, and Ernst, 1954), they did not, however, consider that one particular phage type was more likely to develop resistance. Many other workers subscribed to the point of view that cross infection plays a part in the spread of strains resistant to antibiotics, for example, Clarke, Dalglish, and Gillespie (1952), Blowers, Mason, Wallace, and Walton (1955), Needham and Nichols (1953), and Silberstein (1955). Another study which made it easier to assume that cross infection played a large part in the increasing prevalence of antibiotic resistance was that made by Cairns and Summers (1950). These authors

found that the proportion of open lesions containing penicillin resistant staphylococci increased during the period of hospitalization, whether or not the patients had received penicillin. When a study was made of the incidence of antibiotic resistant strains in fresh lesions first examined in the out patient department of St Bartholomew's Hospital, London, it was found that resistant strains occurred far less frequently than in patients in the hospital, where the incidence of such strains was 60 to 75 per cent. Birnstingl, Shooter, and Hunt (1952) found that 16 per cent of 200 strains isolated from out patients were resistant to penicillin but none was markedly resistant to streptomycin, chloramphenicol, or the tetracyclines. Three years later, Rees, Shooter, and Shawe (1955), on examining another 200 lesions in the same clinic, found that 21.5 per cent had become resistant to penicillin but there was virtually no increase in the proportion of strains resistant to the other antibiotics. Such a slow increase in resistance could be explained in part by the findings of Dowling, Lepper, and Jackson (1953). These authors examined swabs from the noses and throats of 54 patients over a number of weeks before and after discharge from a contagious diseases hospital and also swabs from their household contacts. At discharge 40 of the patients harboured staphylococci, and 35 of these were resistant to penicillin. Four weeks after returning home, however, the number of resistant strains had dropped to 14, which approximated the proportion of resistant strains found in the household contacts. With chlortetracycline the proportion of patients carrying resistant staphylococci, although somewhat lower, followed the same trend. In 7 of the 54 families a hospital strain of staphylococcus was found to have been transferred to a household contact, while the reverse occurred in 10 families that is the strain was transferred from a member of the household to the patient. These findings do not rule out the possibility that resistance of an organism to an antibiotic may be acquired by continued exposure of the organism to the antibiotic over a period of time, but they do show that transference of the staphylococcus from one contact to another is possible and does in fact occur. It seems plausible that resistant strains, however they arise originally, are much more likely to be transferred from one person to another in a hospital, where the resistant bacterial population is high, than in the home, where it is relatively low. Nevertheless the fact that as many as 30 per cent of household contacts were carrying resistant strains is a warning that not only the inmates of hospitals but the general population can become the means of spreading resistant strains. That such a reservoir does already exist is indicated by the tests carried out on 200 blood donors by Rountree, Freeman, and Barbour (1954). Forty nine per cent of these people were found to be nasal carriers of staphylococci, and of these 13.4 per cent carried penicillin resistant strains an increase of 6 per cent over the incidence found 3 years previously. By December 1951 another sampling of 101 nasal carriers among 200 donors showed that the incidence had still further increased to 25.7 per cent (Rountree and Rheuben, 1956). This seems an extraordinarily high incidence. In studying the source of resistant strains, Hinton and Orr (1957) cultured samples taken from the anterior nares of out patients, in patients and staff, and from dust, in Kingston Hospital, Ontario, as well as from students not attached to the hospital. The highest percentage of resistant strains was found among in patients and hospital staff, less in the hospital dust and in out patients, and least of all

among students who were not associated with the hospital. In these, corresponding to the population of blood donors studied by Rountree and Rheuben (1956), the percentage was no higher than 6.5 resistant to penicillin, still less to tetracycline, and there were none resistant to chloramphenicol or erythromycin. Hinton and Orr commented, however, on the incidence of resistance depending on the amount and type of antibiotic most in use.

In one respect strains resistant to penicillin differ from those resistant to other antibiotics. Penicillin resistant strains produce an enzyme which destroys penicillin. This type of organism was found to occur naturally before penicillin was used so widely (Kirby, 1944), but strains in which resistance to penicillin was induced experimentally have not been found to produce penicillinase. This would be an argument in favour of the spread of resistant strains being due to cross infection from the originally rare case harbouring a penicillinase producer. The many studies on the problem of acquired resistance have shown that when an organism is sensitive to penicillin it is sensitive to very low concentrations, seldom exceeding 1 Unit per ml, but when an organism is a penicillinase producer, no definite concentration of the drug will destroy it (Finland and Haight, 1953 and Barber and Burston, 1955). From these findings one may infer that the prevalence of penicillin resistant staphylococci may have resulted from the selective multiplication of the originally rare strains of penicillinase producing staphylococci. The propagation of such strains has been encouraged by cross infection but another factor encouraging their dissemination is the presence in the atmosphere of demonstrable amounts of antibiotic. Work on these lines has been carried out by Gould (1957), who showed that the amount of penicillin recovered per cubic foot of air in rooms of a factory was related to the amount of preparation of penicillin in each room. All strains isolated from the anterior nares of members of the factory were resistant to penicillin, whereas not more than 12 per cent of the population outside the factory were carriers of these strains. Such conditions can be expected to exist in a general hospital where there is much preparation and handling of antibiotic material. By this means of dissemination inhalation of antibiotics can become a common event providing another means of selectively eliminating sensitive strains and allowing those which are resistant to multiply.

Clinical significance of antibiotic resistant staphylococci

So long as a staphylococcus is known to be susceptible to one or another antibiotic, appropriate chemotherapy can be applied. There remain the infections on which no antibiotic can make an impression. That these exist cannot be denied, yet it is not so easy to find examples of irreparable damage caused solely by a completely resistant staphylococcus. Added to the necessity of administering the antibiotic at the earliest possible moment after infection has become apparent, other requirements for successful therapy are the removal of dead tissue such as pus, sloughs, or sequestra, and the ensuring of full access to the infected tissues. When these desiderata are fulfilled, recovery from the infection should follow. When however, all these provisions are not ensured there is ample evidence that resistant staphylococci are associated with serious pathological conditions. For example, there is the rising incidence of infection in clean post operative wounds reported by Howe (1954), the majority of which were due to penicillin resistant

staphylococci: an outbreak of pemphigus neonatorum in a maternity hospital, outbreaks of post operative wound infections in 1955 resistant to penicillin (Shooter Taylor Ellis and Ross 1956) in 1956 resistant to penicillin streptomycin and tetracycline—and after they had been used to chloramphenicol and erythromycin (Shooter Griffiths Cook, and Williams 1957). In nurseries and maternity hospitals a minor epidemic of pemphigus neonatorum due to a penicillin resistant staphylococcus (Gillespie Pope and Simpson 1957) and pyoderma and mastitis resistant to penicillin streptomycin and tetracycline (Wysham Mulhern Navarre LaVeck Kennan and Giedt (1957*a* and *b*) have been described. Fatal broncho pneumonia resistant to penicillin and streptomycin has been described by Gresham and Gleeson White (1957) (see Fig 8*a* and *b*) and by Harley (1957). Loh and Street (1957) and Nordlund and Borden (1958) each described a case of septicaemia resistant to commonly used antibiotics. So serious and so prevalent had these staphylococcal infections become that Wysham and Kirby (1957) conducted a study of all patients admitted to the Cook County Hospital between November 1955 and the end of February 1956 who had staphylococcal infections on admission, acquired it while in hospital or developed it during 60 days after discharge. In this way they found nearly twice as many acquired the infection in hospital as were admitted with it and a sizeable proportion developed it later. The figures were as follows:

| | |
|---|-----|
| Limited with staphylococcal infection | 57 |
| Acquired staphylococcal infection in hospital | 100 |
| Developed staphylococcal infection in 60 days after discharge | 32 |

Judged by the mortality those acquired in hospital were most serious, the number of deaths being 2, 21 and 1 respectively. Twenty six infections acquired in hospital followed operation in which there were 5 deaths. 12 patients developed pneumonia with 8 deaths and in 17 cases with skin infections, burns or decubitus ulcers 2 developed septicaemia and died. In all these cases the staphylococcal infection was considered at least as a contributory cause of death. Only 3 infections were sensitive to all the antibiotics against which they were tested. 26 were sensitive only to bacitracin while others showed varying patterns of resistance, the majority being resistant to penicillin, streptomycin and the tetracyclines.

Complications of therapy

It appears from experience to date that no antibiotic is free from complications of some sort and it is necessary before prescribing treatment for a particular type of lesion to weigh up the advantages and the disadvantages which may follow the use of any particular one. The complications which have received most attention are those due to sensitization, to added infection, fungal or bacterial in origin, to gastrointestinal disorder or to toxic phenomena. In a survey carried out from late 1953 to early 1957 by Welch, Lewis, Weinstein and Boeckman (1957*b*) more than 800 hospitals in the United States were included and over 1,600 attending physicians interviewed regarding the reactions to antibiotics which they had seen. Three thousand four hundred and nineteen case histories were presented of which nearly a third were life threatening. These could be divided into categories according to the type of disorder and the antibiotic most usually associated with it. They



FIG. 81. Smear of tracheal pus from a case with staphylococcal bronchopneumonia, whose primary cause of death was a mediastinal tumour and a head injury. The patient had previously received sulphadiazine, penicillin and streptomycin.

- b. Stained section of lower lobe of lung from the same case showing a bronchiole filled with pus and surrounded by alveoli filled with red cells.
(From Gresham and Gibson White. *Lancet*, 1957, 1: 631.)

were anaphylactoid reactions—809 cases for which penicillin preparations were mainly responsible, superinfections (mainly of the gastro intestinal canal)—107 cases in which the tetracyclines played the major role blood dyscrasias—46 cases in the great majority of which chloramphenicol alone or with other antibiotics was implicated, skin reactions—70 cases and angio neurotic oedema—38 cases in which penicillin again was the major factor

Sensitization

Sensitization, in the form of rashes urticaria, angioneurotic oedema serum sickness, or anaphylaxis has been most commonly met with after the administration of penicillin Allergic reactions to novobiocin have also been frequently reported in its early clinical trials The extraordinarily widespread use of penicillin may be responsible for this, the fact that it is still most frequently given parenterally or the possibility that it has in its composition some particularly strongly sensitizing factor Kekwick (1956) considered that the frequency of severe reactions in this country was of the order of 2 per cent during the first 9 years of the clinical use of penicillin, but claimed that in the next 2 years in 15 per cent such reactions had been reported Harris (1954) also commented on the increasing frequency of anaphylactic shock following penicillin yet when Welch *et al* (1957 *b*) stated that 2½ million lb of antibiotics 38 per cent of which was penicillin were produced in the United States in 1956 representing a steadily rising annual total since 1953, the increasing frequency of reactions is not surprising Fortunately, these reactions seldom appear on first exposure to penicillin and they are more likely to occur in patients who have a past history of allergy, such as asthma, hay fever, or skin reactions It is possible however, that sensitization may be induced in people from the ingestion of dairy products From several surveys of milk samples, the last completed in January 1956, Welch (1957) reported that 3 to 11 per cent of milk samples contained up to 0.55 Unit per ml of penicillin—derived from cows treated with penicillin for mastitis Although no proven case of allergy had arisen from this source, the possibility cannot be overlooked The clinician can be thus forewarned and can choose another antibiotic more suitably given by mouth if there is any doubt about the possibility of producing a sensitization reaction

Sensitization reactions have also been recorded occasionally with antibiotics other than penicillin Monnet, Froment, and Traeger (1950), for example describe the case of a patient with subacute bacterial endocarditis in whom penicillin failed to sterilize the blood The patient was then given 4 G of chloramphenicol in 2 hours Several hours later the patient collapsed, became dyspnoeic, pale, and cyanosed, and died on the same day At post mortem no intra cardiac thrombosis was found to account for death, but it is possible that the gross vegetations on the aortic valves might have temporarily blocked a coronary artery while the heart was in diastole Nevertheless there was no sign of the myocardium being altered in any way Pulaski (1950) and Finland and Weinstein (1953) mention the occurrence of sensitization reactions after the administration of chloramphenicol or the tetracyclines They have also followed the use of streptomycin

Gastro intestinal complications

These have received considerable attention since the introduction of the

tetracyclines into clinical medicine. They are not, however, confined to the tetracyclines but may arise after the administration of any antibiotic. For example, Pulaski (1950) described gastro intestinal complications following the administration of chlortetracycline, oxytetracycline, and 'other antibiotics', Baca, and Pitzurra (1955) and Choremis, Tsengi, and Economou Mavrou (1954) following penicillin, streptomycin, chloramphenicol, and chlortetracycline, and Cunningham and Beaven (1955) following chlortetracycline, penicillin, streptomycin and oxytetracycline, and erythromycin. It is questionable whether this complication begins as a direct irritation of the mucosa of the gastro intestinal wall on which an infection is superimposed, whether it is due at first to sensitization, or whether the infection is the immediate cause. Some investigators attribute the gastro intestinal irritation to vitamin deficiency caused by the removal from the gastro intestinal canal of organisms which are responsible for the synthesis of certain vitamins. This is not proven according to Finland and Weinstein (1953), except in the case of chlortetracycline, the administration of which causes increased loss of riboflavin, but no further evidence in favour of the hypothesis has been found by Goldsmith (1956). In many cases treated by the tetracyclines the stools become bulky, soft in consistency, and have a peculiar odour. These changes, however, are not of very serious consequence and stop with the discontinuance of the drug. Other patients have more severe symptoms, with persistent watery diarrhoea leading to dehydration and collapse. From the faeces of these latter patients various bacteria have been isolated, for example, *Staph aureus*, proteus, or pseudomonas (Cook, Elliott, Elliot Smith, Frisby, and Gardner, 1957, Finland and Weinstein, 1953, Girard, Fraisse, and Simon, 1954). In other cases the onset is fulminating and death, in spite of supportive treatment and even erythromycin therapy, may take place within 24 to 36 hours. Such cases have been described by Fowler (1955), Frame and Short (1955), Frederiksen (1956), Nador and Câmara Lopes (1957), Scholz (1955), Spearo (1954), Squires and Foote (1954), Terplan, Paine Sheffer, Egan, and Lansky (1953), Thaysen, Eriksen, Fischermann, and Knudsen (1955), and Todd and Hopps (1955). The severe complication is generally ascribed to an enterotoxin producing staphylococcus. In support of this hypothesis Surgalla and Dack (1955) isolated staphylococci from the faeces of 33 patients with enteritis and tested the products of the organisms on monkeys. They found that 30 of the strains produced enterotoxin. Although the occurrence of this complication followed the introduction of the tetracyclines into medicine, there have been instances where the administration of other antibiotics has been a forerunner of the condition. For example, Frame and Short (1955) describe a patient with enteritis following the removal of a ruptured gangrenous appendix. This patient at first received only penicillin and streptomycin intramuscularly. When, however, after a second operation these drugs were replaced by oxytetracycline, the patient's condition deteriorated and death followed on the day after operation in spite of the administration of erythromycin, intravenous fluids and yoghurt. Staphylococci in this patient were isolated from the throat, intestine, blood, and viscera, and were resistant to penicillin, chlortetracycline, and oxytetracycline. Fowler (1955) also describes 3 cases in which severe diarrhoea associated with resistant staphylococci in the intestine occurred after operation. In these patients the only chemotherapy given before the complication

occurred was streptomycin and procaine penicillin. Similarly, one of the patients described by Todd and Hopps (1955) had only received penicillin and streptomycin before a partial gastrectomy which was followed by the

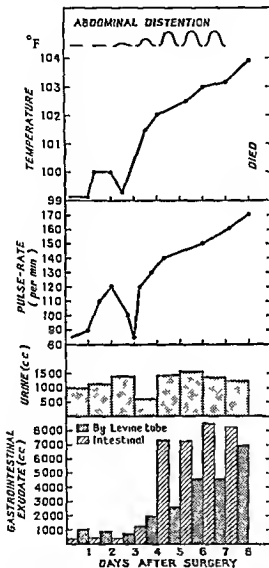


FIG 9a Pulse, temperature, and output chart of a patient following subtotal colectomy for ulcerative colitis. The characteristic signs of staphylococcal enteritis i.e. abdominal distension, elevation of pulse and temperature, oliguria and the outpouring of gastro intestinal exudate appeared on the 3rd to 5th day. The patient died on the 5th post operative day, the disease unrecognized and untreated.

(From Turnbull, *J Amer med Ass* 1957, 164, 756)

enteritis. It is of interest to note how many of the examples given above have followed abdominal operations. Fig 9a and b shows the increasing abdominal distension, pulse rate, temperature, and disturbance of fluid output produced by this type of enteritis and, in Fig 9b, the effect of suitable early management. Fig 9c and d shows the macroscopic and microscopic appearances

of the complication in the gut. A case could therefore, be made out that the operations were responsible for the onset of the enteritis. Dixon and Weismann (1948) describe 23 cases brought to autopsy between 1940 and 1947, a time when little clinical use had been made of antibiotics. It is, however, worthy of note that where the predominant organism in the alimentary tract

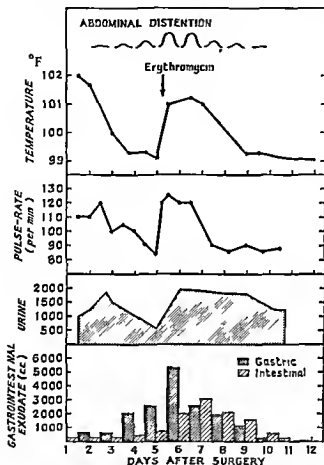


FIG. 95. Post operative course of ulcerative colitis in a man aged 42 treated with oxy tetracycline streptomycin penicillin salicylazosulfapyridine and cortisone for 1 month before subtotal colectomy. Treatment of diarrhoea—antibiotics discontinued and erythromycin given intramuscularly. Result—much clinical improvement in 12 hours (From Turnbull *J Amer med Ass* 1957 164, 756)

has been isolated it has been found to be resistant to all the antibiotics with which each patient had previously been treated.

Complications arising in other parts of the alimentary tract have been described by Verhuss and Hoffman (1951), Harris (1954), and Turell and Maynard (1954). Turell and Maynard collected 136 such cases. These included pruritus ani (with or without accompanying diarrhoea), or pruritus with anogenital soreness. One hundred and twenty five of these cases followed the use of chloramphenicol or the tetracyclines, but 11 followed the use of erythromycin. The fact that none followed penicillin or streptomycin may simply be an indication of the small use made of these 2 antibiotics in

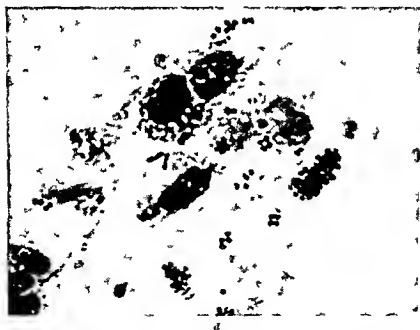
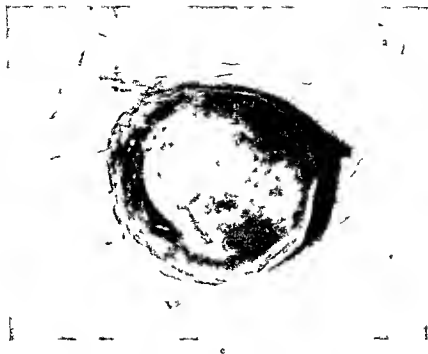


FIG. 9. Pseudomembrane seen on local opening in a case of staphylococcal enteritis.

d. Stained smear from pseudomembrane showing pus cells and micrococci.
(From Turnbull *J. Amer. Med. Ass.* 1937 164 756)

the clinic of these workers. Similar problems in treatment have been described by other workers to whom reference has been made.

Superadded bacterial infection

Infection due to organisms insusceptible to the antibiotic being used has been observed since the early days of penicillin therapy. The superadded infection was readily recognized for the bacteria were usually Gram negative rods and easily distinguishable from the Gram positive bacteria which were mainly responsible for the original infection being treated (Florey and Cairns, 1943). At that time these Gram negative organisms were not considered to be serious invaders of tissue, but at a later date when some of them were found to resist the action of the tetracyclines, especially in urinary tract infections, they were considered to be a much more serious problem. Still later, antibiotic resistant staphylococci were found to be associated with typical clinical scarlet fever in patients being treated for pertussis (Finland, 1951). Finland, Jackson, Kass Haight, and Womack (1951) also found resistant staphylococci associated with the appearance of grossly purulent sputum in some patients being treated for respiratory tract infections. Hofer and McCaskey (1954) observed relapses associated with the appearance of resistant organisms in infections undergoing antibiotic therapy. These authors questioned whether the appearance of the resistant organisms was due to their selective growth in the presence of the antibiotic or whether some alteration of the tissue response had been produced by the disappearance of the primary invaders. Keefer (1951), however, was of the opinion that the change in the flora during the administration of an antibiotic was independent of its effect on the specific organism responsible for the original infection, but depended on the survival of organisms originally present but insusceptible to the drug on the development of resistance in these strains, or on the introduction of new strains from outside.

The incidence of superadded infection in medical wards devoted to infectious diseases was studied by Weinstein, Goldfield, and Chang (1954). These authors observed 3 095 patients passing through their hospital and estimated the incidence of superadded infection to be 2.19 per cent. However, this incidence was as high as 12 to 15 per cent in patients who were treated with streptomycin, chlortetracycline, or oxytetracycline. Usually the superadded infection occurred at the site of the primary disease but in young children it sometimes also appeared in the lungs or middle ear. The responsible bacteria were usually staphylococci, *Haemophilus influenzae*, or coliforms. Sometimes the secondary infection was more serious than the original disease. This raised the question whether antibiotics should be used in conditions such as measles or pertussis. Bronchopneumonia, urinary tract infection, and meningitis had in some cases been superimposed on the primary disease. This was noted as early as 1947 (Weinstein) when only penicillin and streptomycin were in widespread use. In 1955 this author had found that as many as 30.4 per cent of children admitted with measles, and having been previously treated with antibiotics, had developed some secondary bacterial disease—twice the proportion of those who had received no previous antimicrobial therapy.

Fungal infections Geiger, Wenner, Axilrod, and Durlacher (1946) drew

attention to the fact that a lesion undergoing antibiotic treatment might become infected with *Candida albicans*. This infection was observed in one patient with endocarditis who had received 2 courses of penicillin. When death supervened *Candida albicans* was cultured from the heart valves. Cawley (1947) also reported on a child of 8 years who had had repeated exacerbations of pulmonary aspergillosis since she was 2 weeks old. When penicillin and sulphonamides were adopted for her treatment, dissemination of the condition occurred throughout the body, resulting in death. Neither of these reports constituted unequivocal evidence that the fungal infection was instigated by the antibiotics, but these cases none the less gave cause for alarm. In a patient with influenza being treated by penicillin, Grekin, Cawley, and Zheutlin (1950) noticed the appearance of thick purulent sputum together with patchy exudate on the oral mucosa. This marked the beginning of a fatal disease, and these authors considered that the administration of the penicillin was responsible for the widespread dissemination of this fungal growth. Reiches (1951) next demonstrated monilial overgrowth in the perianal region of a patient receiving chloramphenicol, chlortetracycline and oxytetracycline, and Shrigley and Rowson (1951) reported a heavy infection of the urine with *C. albicans* following treatment of cystitis with penicillin, streptomycin, and chloramphenicol. The most common site for monilial overgrowth is, of course, the mouth. Woods, Manning, and Patterson (1951) described the occurrence of sore mouth and throat, hairy tongue, and clinical thrush 24 to 72 hours before the fungous infection became manifest. This sometimes appeared as areas of whitish exudate and involved the soft palate, uvula, aryepiglottic folds, pyriform fossae, and the oesophagus. Such manifestations have been seen in 20 patients who received penicillin or chlortetracycline as troches or sprays, or penicillin and streptomycin parenterally. Besides invading the mouth, the fungal infection is apt to spread down into the lungs and infect pulmonary cavities. Observations of this sort have been made by Abbott, Fernando, Gurling and Meade (1952) and Stenderup, Bichel, and Kissmeyer-Nielsen (1956). It is also possible, under the influence of antibiotics, for the fungi to invade the gastro intestinal canal (Neuhauser, 1954) and, as mentioned above, the blood and valves of the heart (Zimmerman, 1950). No antibiotic in general use at present is free from this possible complication, but nystatin used specifically for the suppression of monilia seems to be a successful antidote.

Toxic manifestations

Fortunately these are uncommon in the widely used antibiotics. No toxic phenomena have yet been associated with penicillin, although it has been reported by Bateman, Barberio, Grace, Klopp, and Pierpont (1952) that death ensued in a dog when the serum concentrations produced experimentally reached 4 000 Units per ml. The cause of death, however, is not stated. Changes in the kidneys have been reported following bacitracin and polymyxin B and neomycin. Neurotoxic manifestations are common with streptomycin, dihydrostreptomycin and neomycin, the drug in the first instance attacking the vestibular branch of the 8th cranial nerve and, in the case of dihydrostreptomycin and neomycin, the auditory branch. Deleterious

effects on the central nervous system amounting sometimes to convulsions have followed administration of cycloserine, transient ataxia and paraesthesiae have been noticed with polymyxin B, but these have not been serious in patients in whom the dose was kept down due to the appearance of renal symptoms. Changes in liver function have been described after the administration of high doses of the tetracyclines or chloramphenicol (Yesner and Kunkel, 1951). There have been many studies on the alteration of clotting, prothrombin, and bleeding times following the administration of various antibiotics, but effects on blood clotting only occur at concentrations well above those required for therapeutic purposes (Seegers, 1951). Leucopenia and agranulocytosis have followed administration of streptomycin, chloramphenicol, and chlortetracycline, and aplastic anaemia and bleeding syndromes following the use of chloramphenicol and even oxytetracycline. Deaths have been reported following chloramphenicol, although it cannot be stated unequivocally that these were due to the antibiotic. Their degree of toxicity is such that only local applications are justifiable in the administration of tyrothricin and the amphotericins, oral administration and local application are justifiable in those toxic antibiotics which are not readily absorbed from the gastrointestinal canal such as framycetin, bacitracin, polymyxin, and neomycin, and parenteral administration can be given with suitable precautions for infections which respond to no other drug in the case of bacitracin, polymyxin, and neomycin. The toxicity of the antibiotics such as the tetracyclines, chloramphenicol, erythromycin, carbomycin, apiramycin, and novobiocin is so low that their ready absorption through the gastrointestinal canal enables them to be administered by mouth with little fear. Those which are not so absorbed—vancomycin and ristocetin—appear as yet to have few untoward effects from intravenous administration.

Of the remainder, clinical trials have as yet been too few to recommend any with assurance for treatment.

Prevention and treatment of complications

Obviously, if it is possible to avoid these various complications by wise choice of an antibiotic, this is very much to be preferred to attempting to treat the complications after they have appeared. Except for prophylaxis for rheumatic subjects against haemolytic streptococcal infections and the few days of pre-operative preparation before bowel surgery, it is doubtful whether any routine prophylaxis with antibiotics is justifiable in view of the widespread opportunity it gives for the development of infection with antibiotic resistant organisms. In surgical wards, at least, limitation of antibiotics to therapeutic measures would reduce their use to a fraction of the present amount.

Sensitization

This mainly concerns penicillin. Since these reactions very seldom arise in patients on the first administration of the drug, it seems safe to give at least a first course of penicillin. However, no one should receive penicillin therapy before inquiry has been made into a possible history of allergy and whether there has been previous treatment with the antibiotic. If previous treatment with penicillin has been accompanied by any type of reaction it is preferable

to use another suitable antibiotic, or, if penicillin G has been used previously, penicillin O can be tried in a small dose first, followed later, if no untoward reactions occur, by therapeutic doses. With the introduction of penicillin for oral use, this route would seem to be another means of circumventing sensitization reactions. Dihydrostreptomycin fortunately can replace streptomycin in nearly all instances.

Once a reaction has occurred the antibiotic should be discontinued immediately and, if possible, a substitute used. If the reaction is limited to the skin, antihistamine drugs should benefit the patient, but when a delayed type of reaction occurs which involves joint, glandular, or renal manifestations, the antihistamine drugs are not of any value. In these cases, cortisone or corticotrophin are sometimes of value. There is also the possibility that penicillinase may control the reaction providing penicillin is the exciting factor. When the reaction is so severe as to be considered anaphylactic, urgent measures should be taken. Adrenaline (0.1 ml of 1:1,000 solution) should be given subcutaneously and followed by further subcutaneous injections every few minutes until 1 ml has been administered, provided the pulse rate does not rise too rapidly. Occasionally tracheotomy is required when oedema of the glottis and epiglottis occludes the air way. The injection of adrenaline and tracheotomy are emergency measures, and the necessary apparatus should be at hand for immediate use if required. When the reaction is less severe, intermittent injections of adrenaline, or ephedrine by mouth (50 mg 3 times a day), can be given. When the patient has obtained relief, cortisone or ACTH treatment for some days may help to relieve the reaction.

Recently penicillinase, an effective inactivator of penicillin, has been introduced as therapy by Becker (1956). Sensitization reactions which persist for days or weeks may be held in check by more rapid methods until penicillinase has had time to eliminate all residual penicillin in the body. A Unit of penicillinase is that amount which inactivates a Unit of penicillin in a given time and a few thousand Units repeated in several hours will inactivate many times this amount of penicillin. In experimental work the blood can be cleared of penicillin within an hour but in clinical practice, when penicillin is already in the body, 24 hours to 4 days are required to complete the effect (*Lancet*, 1957). During this time more rapidly acting preparations can be used to counteract the allergic manifestations until the patient experiences more permanent relief.

It is also possible, when the reaction has subsided, to desensitize the patient by increasing doses of the antibiotic. Desensitization should be begun with the smallest amount which produces no reaction, given intradermally or by mouth. When an antihistamine is added to each injection it has been found possible to hasten the desensitization (Balme and Dormer, 1954). With penicillin at least, there are phases in the allergy, and it is sometimes possible, without any preceding desensitization, to give another course of the drug without producing a reaction. However, this is a risk that would hardly be run except in the most special circumstances.

Gastro intestinal disturbances

Obviously the lowest dose compatible with effective treatment is the desideratum when administering antibiotics by mouth. To prevent these

disturbances several possible lines of action have been tried. Firstly, preparations have been used which enhance the absorption of the tetracyclines and so enable one to prescribe a lower dose. Such a preparation was described by Kaplan, Dickeson, Hubel, and Buckwalter (1957). This was a tetracycline phosphate complex, a crystalline salt, soluble in water to the extent of 3 to 5 mg per ml but much more so in artificial gastric juice. Its antibacterial range was identical with tetracycline but its activity only 75 to 80 per cent of that of the hydrochloride. Equivalent doses, when described in terms of the activity of the hydrochloride, produced almost twice the concentration in the blood serum of human subjects. These claims were substantiated for tetracycline, oxytetracycline, and chlortetracycline by Welch and his co-laborators (1957 *a, b, c, e, and f*), who also showed that a mixture of antibiotic with sodium hexametaphosphate was at least as effective as the complex phosphate salt. Correspondingly higher levels were also found by Pulaski and Isokane (1957 *b*) in certain body fluids such as bile and prostatic fluid. Clinical trials¹ showed that the complex or mixture was therapeutically satisfactory in a dose equivalent to half that in common use with the antibiotics alone, but without control series it was difficult to decide whether the incidence of gastro intestinal reactions had been lowered. Undoubtedly they still occurred, possibly less frequently. An agent still better able to facilitate absorption of the tetracyclines was citric acid (Dearhorn, Litchfield, Eisner, Corbett, and Dunnett, 1957; Sweeney, Hardy, Dornbush, and Rueggsegger, 1957). So far no clinical trials of a mixture of this and a tetracycline have come to the author's notice. One disadvantage of the commercially prepared metaphosphate preparation was the presence of an excipient (dicalcium phosphate) which depressed absorption. However, even when this was omitted from trial preparations, Welch and Wright (1957 *a*) still found that the metaphosphate did not enhance absorption to as great a degree as did citric acid.

Another method of dealing with gastro intestinal disturbances depends on the belief that these are caused by the supervention of infection due to antibiotic resistant bacteria. These are kept in check by the normal flora of the gut but when these in their turn are held in check by antibiotics, a free field is left for the resistant pathogens. On the assumption that certain saprophytic organisms can adopt the function usually practised by the natural intestinal flora, Gordon, Macrae, and Wheeler (1957) followed the example of Lob and Baker (1955) and used in clinical trials a preparation containing *Lactobacillus acidophilus* to which were added complete growth factors and nutrients known under the trade name of *Enpac*. The lactobacillus had first been made resistant to penicillin, streptomycin, chloramphenicol, neomycin, the three tetracyclines, and erythromycin. These workers studied the effect of administering the preparation to children (3 G 4 times a day during and for 4 days after tetracycline). Tetracycline was given in a dose of 10 mg per kg of body weight daily for 5 days. On making bacteriological examination of the stools of 66 patients, of whom alternate ones received *Enpac*, Gordon *et al* found that staphylococci initially increased in numbers but, in those receiving *Enpac*, simultaneously with the increase in lactobacilli, there was a pronounced drop in the staphylococcal count. There was no

¹ Cronk and Naumann (1957). Rein and Fleischmajer (1957), Prigot, Shidlovsky, and Felix (1957). Putnam (1957).

demonstrable effect on enterococci or coliform bacilli. These results were satisfactory from the bacteriological point of view, and from the clinical aspect there were no bowel or other side effects. Nevertheless, taking into consideration that treatment only lasted for 5 days, the absence of irritation of the gastro intestinal canal in either controls or treated cases leaves the test lacking in corroborative clinical evidence of the beneficial influence of *Enpac*.

Insusceptible superadded infection

The means used to prevent this depend largely on the interpretation of how the infection is produced. If it is a matter of the selective action of the antibiotic allowing organisms not originally evident in a lesion to grow more rapidly once the primary invader is controlled, it seems possible to envisage treatment with a succession of antibiotics effective against each organism in turn. If, however, superadded infection is a matter of cross infection of a resistant strain from one member of a ward to another, much stricter precautions than are now practised should be taken to prevent this occurrence. As resistant staphylococcal infection is the most serious problem in this respect, it is of great interest to know how far cross infection can be prevented by measures already known, such as the isolation or spacing of beds, the wearing of impermeable masks by the nursing and medical staff, the oiling of blankets, floors, and walls, and the use of ventilation fans to draw bacteria laden air downwards out of reach of the lesions of the patients. Where a surgical ward is concerned, no touch technique for the dressing of wounds, involving a team of dressers each with a special function, has been in practice in several units where cross infection in the ward has not been prominent. In the theatre, however, where large open wounds are made, the opportunity for the introduction of pathogenic bacteria into the tissues is considerably greater. Where a resistant and pathogenic staphylococcus has gained access to a hospital and has become prevalent among the hospital staff, cross infection is particularly difficult to control. Although this problem is not insuperable, it is beyond the scope of this volume.

The third explanation for the supervention of resistant infection, particularly in the case of the staphylococcus, is the development of resistance to the antibiotic *in vivo*. To prevent these cases one must consider whether there is sufficient evidence of effective synergism between antibiotics to render the combined administration of 2 or 3 justifiable in the circumstances. Jawetz and his collaborators have given much attention to this problem. These authors have found that antibiotics *in vitro* may act independently of one another, may antagonize each other, or may act synergistically. As an example of the clinical application of synergism, Jawetz, Gunnison, Bruff, and Coleman (1952) described the action of penicillin and streptomycin on enterococci, and of streptomycin and chlortetracycline on brucellae. However, Jawetz has given warning that the same combination of drugs may be synergistic in certain concentrations and antagonistic in others (Jawetz, 1953). He has concluded that those drugs which are bactericidal in their action are more likely to be synergistic than those which are only bacteriostatic. Thus penicillin, streptomycin, bacitracin, and neomycin can usually be depended on to destroy bacteria more readily when 2 are used together.

rather than one. Chloramphenicol, the tetracyclines, and erythromycin are more often bacteriostatic at concentrations used in therapy, and their effect in combination cannot be predicted unless bactericidal tests are carried out *in vitro* as a preliminary to clinical application. These conclusions are summarized in a paper by Jawetz (1954). Many papers have been written on the subject of synergism and antagonism, but the results are by no means concordant. Eventually the justification for using a combination of antibiotics shown to be bactericidal *in vitro* must be their successful clinical application. A simple test for choosing combinations has been described by Elek, Hilsen, and Jewell (1953). A sterile velvet pad is pressed on to the agar culture containing the antibiotics to be tested and transferred to another culture plate free of antibiotics. If, after incubation for 18 to 24 hours, the pad or the plate remains free of bacteria, one may conclude that the great majority of the organisms have been killed. These workers tested 65 strains of common pathogens against 3 pairs of antibiotics and found that penicillin with either chloramphenicol or chlortetracycline generally showed antagonism, while streptomycin with chloramphenicol usually showed synergism. The criticism to be offered of this technique is that the requisite concentrations for each antibiotic are not known. Another test for assessing the effectiveness of combinations in fluid media is that described by Jawetz, Gunnison, Coleman, and Kempe (1955). On the other hand the concentrations of the antibiotics in the body after each dose fluctuate so markedly that the most effective concentrations might well be present only momentarily after each dose. Much work has been done by Jones and Finland (1957) on the effect of giving two antibiotics together on raising the inhibitory activity of the plasma. With tetracycline and oleandomycin, erythromycin, spiramycin, or penicillin V they have found little evidence of enhancement against certain susceptible or insusceptible pathogens. Fig. 10 shows how little effect there was on *Strep. 98* when penicillin V and tetracycline were given together in a total dose equivalent to that of penicillin alone. Fig. 2a and b on p. 55 and Fig. 10 are examples of how little this enhancement of activity against a susceptible streptococcus or staphylococcus can be demonstrated. Similar findings held for a pneumococcus. Evidence of synergism in the clinic has been most readily obtained in the treatment of bacterial endocarditis. Hunter (1950) and Robbins and Tompsett (1949) both demonstrated *in vitro* synergism between penicillin and streptomycin against *Str. faecalis* and were able to cure patients suffering from this infection by a combination of the antibiotics. Garrod (1953) cites 4 cases in each of which the responsible organism was a *Str. faecalis* inhibited by concentrations of penicillin, chlortetracycline, and chloramphenicol, which, although not very low, could easily be reached in the serum. However, to inhibit the organism somewhat more streptomycin was required than could be given without risk of producing vestibular disturbance. None of these patients responded to the antibiotics to which the streptococcus was most susceptible, namely chlortetracycline or chloramphenicol. In all 4 patients the infection was eventually controlled by penicillin and streptomycin. A case undergoing prolonged treatment with a succession of different antibiotics was described by Balme and Dormer (1954). This patient, whose infection was also due to *Str. faecalis*, received in succession penicillin (12 million Units daily) for 6 weeks, chlortetracycline (2 G daily), and oxytetracycline (6 G daily) each for 3 weeks, and then the last 2

antibiotics simultaneously for 7 days. Chloramphenicol was then substituted for chlortetracycline but without effect on the fever, embolism, or the general condition. In spite of the organism being inhibited *in vitro* by quite low concentrations of chlortetracycline and oxytetracycline and only by somewhat high concentrations of penicillin and streptomycin singly, when the 2 latter antibiotics were used together, the combination sterilized the inoculum in 24 hours. Treatment with 2.5 million Units of penicillin 3 hourly and 0.5 G. of streptomycin 6 hourly by intramuscular injection for 6 weeks

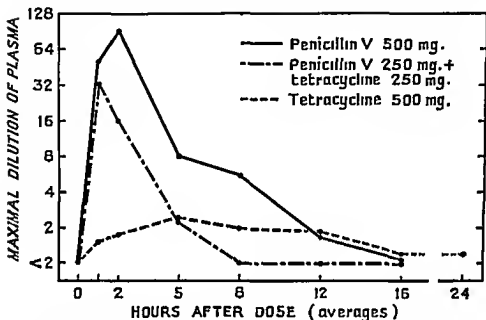


FIG. 10. Inhibiting values of plasma for *Str. 98*—sensitive *in vitro* to 0.945 μg per ml of penicillin V to 0.35 μg per ml of tetracycline and to 0.09 μg per ml of equal parts of each—following ingestion of 500 mg. of either antibiotic or of 250 mg. each of tetracycline and of penicillin.

(From Jones and Finland *New Engl J Med* 1957 25b, 859.)

ended in the patient's recovery. Tests of the blood cultures were repeatedly sterile, and no recurrence had occurred over the following 6 months. Penicillin and streptomycin were also found by Geraci and Martin (1954 b) to be the most reliable combination for enterococcal infection. A few workers, however, have reported other combinations which were successful after previous antibiotic therapy had failed. Staphylococcal endocarditis has been notoriously difficult to treat successfully, but Jawetz, Gunnison, and Speck (1951 b) succeeded in dealing with 1 case in which a coagulase negative staphylococcus was responsible. Although no clinical response was made to chlortetracycline and penicillin when used singly, and the staphylococcus, when isolated, was found to be resistant to more than 20 μg per ml. of every antibiotic tested, yet 10 μg per ml. of streptomycin and of oxytetracycline used simultaneously was rapidly bactericidal *in vitro*. When the patient was treated with 2 G. of oxytetracycline daily intravenously and 3 to 4 G. of streptomycin by intramuscular injection every 24 hours for 10 days, he recovered. Tompsett (1953), in reviewing his experience in the treatment of

bacterial endocarditis, found that his best results in staphylococcal infections followed a combination of penicillin and dihydrostreptomycin, but control of the infection could sometimes be obtained with a combination of dihydrostreptomycin and a tetracycline or chloramphenicol, or of penicillin and bacitracin. More recently tests *in vitro* have indicated that novobiocin and vancomycin in combination with one another or with neomycin or bacitracin kill at a greater rate than either of the first 2 antibiotics alone (Jawetz, Bertie, and Sonne, 1957).

Fisher, Wagner, and Ross (1955) report a case in which erythromycin, together with penicillin, seems to have played a part in recovery. In 38 cases treated by these authors at the Johns Hopkins Hospital between 1933 and 1949 it was found that, irrespective of the concentration of penicillin required to inhibit the infecting organism, massive doses of this antibiotic (for example, 8 to 24 million Units per day) given together with up to 3 G of erythromycin daily for as long as 5 weeks, appeared to be most successful against staphylococcal infections. These workers also added a tetracycline or chloramphenicol for good measure, but it is difficult to evaluate the latter drugs in the combination. Bunn and Cook (1954) found streptomycin and chloramphenicol effective in endocarditis due to a Gram negative organism. Loewe, Cohen, and Eiber (1953) studied a case due to *Str viridans* which had been treated over 7 months with chlortetracycline, penicillin combined with chlortetracycline, and penicillin combined with chloramphenicol. After admission the patient received penicillin combined with oxytetracycline and carinamide, oxytetracycline combined with dihydrostreptomycin, and procaine penicillin combined with probenecid and 100,000 Units of bacitracin daily for 4 doses. Seven days after bacitracin had been added to the therapy, all antibiotics were discontinued. The blood culture had become sterile, the temperature soon fell, and there were no signs of reactivation during the following 24 months. The 2 drugs which were found to be bactericidal *in vitro* were penicillin at a concentration of 12 Units per ml and bacitracin at a concentration of 0.75 Unit per ml. While it is not legitimate to attribute recovery in this case to the combination of these 2 antibiotics only, it is to be noted none the less that recovery took place while the patient was under treatment with antibiotics known to be bactericidal. Fig. 11 is the chart of the patient during his stay in the Brooklyn Jewish Hospital after the many months of ineffectual treatment with penicillin and/or chlortetracycline.

Another attempt to apply laboratory findings to the clinic was made by Flippin and Eisenberg (1954). In an investigation lasting over 3 years these authors studied the bacteriostatic and bactericidal effects of certain antibiotics, singly and in combination, on different organisms. Both bactericidal and bacteriostatic effects could be demonstrated with chlortetracycline, oxytetracycline, and penicillin singly, and these effects were enhanced when the drugs were given together. It was found also that chlortetracycline, oxytetracycline, and chloramphenicol behaved synergistically towards *Klebsiella pneumoniae*. Results from combined treatment of pneumococcal pneumonia and staphylococcal infections could not, however, be considered better than those obtained by intramuscular injection of procaine penicillin alone, but in 12 patients with refractory urinary tract infections in which *Klebsiella pneumoniae* was the responsible organism, complete recovery was produced in 5 cases by a combination of the drugs. Better results were obtained by

Smith and Smith (1954) with infections of the urinary tract due to *Bact coli*. With a combination of 0.5 G of oxytetracycline 4 times daily by mouth, and 1 G of streptomycin twice daily by injection, clinical improvement occurred within 12 hours in 27 patients, with infections arising from obstetrical or gynaecological conditions. This was less than half the time taken to produce clinical improvement in another 25 patients treated with oxytetracycline alone.

In none of these multiple therapies does the possibility of antibiotic antagonism seem to have been considered. There is in fact one instance reported by

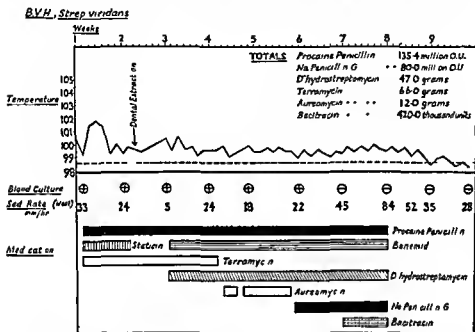


FIG. 11 Chart of a patient with endocarditis due to *Str. viridans* after having received 17 months' treatment with penicillin and chlortetracycline separately and together, and penicillin and chloramphenicol. Blood cultures became and remained sterile after he was on treatment with penicillin, dihydrostreptomycin and bacitracin.

(From Loewe, Cohen, and Eiber, *Antibiot and Chemother* 1953 3, 681)

Lepper and Downing (1951) in which chlortetracycline and penicillin seem to have acted antagonistically to one another. This was in pneumococcal meningitis. For this condition these workers had previously adopted the practice of administering as much as 1 million Units of penicillin intramuscularly every 2 hours in order to obviate the necessity of intrathecal injection. With this therapy 13 of 43 cases of this disease had died, or less than one third of the cases. The newer antibiotic was tried, together with penicillin, which was given in the same dose and in the same way. Fourteen patients were treated by penicillin alone and alternately with 14 by the combination of drugs. It was found that 11 of those treated with the 2 antibiotics died, or nearly four fifths of the cases. Irrespective of the nature or gravity of the case, the mortality amongst patients treated with the 2 antibiotics was always higher than in those treated by penicillin alone. It is possible that this difference might have been due to coincidence, but the risk of an antagonism between the two drugs does not seem worth running.

The treatment of fungal overgrowth

In the mouth or about the anus this complication is not very difficult to deal with, provided it is recognized early and antibiotic therapy is stopped at once. It is, of course, possible to treat the condition with nystatin, and this has been recommended by Sternberg, Tarbet, Newcomer, Huddleson, Weir, Wright, and Egeberg (1953). These authors were able to reduce the monilial population in the faeces at the same time as a tetracycline was being administered. So effective was nystatin that it was recommended for prophylactic use, as a concomitant of all oral therapy with tetracyclines, chloramphenicol, or neomycin. Experience in the past, however, has revealed the calamitous consequences of such widespread use of an antibiotic, and it would seem wiser to withhold the drug until some evidence of fungal infection is present.

Toxic effects

The indicated course of action when an antibiotic is producing toxic effects is discontinuance of the antibiotic. There are, however, occasions when no other antibiotic but the one producing the toxic effect is active in the infection being treated. Such cases justify the parenteral use of bacitracin, neomycin, or polymyxin B or E if the dose is limited and a careful watch is kept on renal function and, in the case of neomycin, hearing. If it is impossible to find any other suitable treatment for a patient whose kidney function is not normal, treatment with neomycin may be given parenterally, but only with the utmost caution.

Adjuvant effect of special agents - the corticosteroids

Of these, much the best known are the corticosteroids. Since the original observations of Hench, Kendall, Slocumb, and Polley (1956) on the powerful effect of pituitary ACTH and of cortisone on the manifestations of rheumatoid arthritis, there have been many reports on the administration of these substances together with antibiotics. Sometimes the results were dramatic, at others they were catastrophic or at least unfortunate, as for example the development of purulent meningitis or an extensive abscess during administration of corticotrophin or cortisone together with procaine penicillin. It has been argued that though, without antibiotics, there is ample evidence that the barriers of the host against infection are lowered by administration of corticosteroids, the giving of an antibiotic simultaneously and in sufficiently high doses may overcome this adverse effect. In this manner Herrell (1957) advocated the use of combined therapy in a few bacterial diseases, viz. tuberculous meningitis, a Waterhouse-Friderichsen syndrome in meningococcal septicaemia, typhoid fever, brucellosis, and severe tetanus, quoting authorities for his conclusions. Unfortunately, as Kass and Finland (1957) have ably pointed out, the infrequency of controlled trials has prevented this argument from being put to the proof. One such trial in pneumococcal pneumonia carried out by Wagner, Bennett, Lasagna, Cluff, Rosenthal, and Murick (1956) gave little indication as to whether the prognosis had been improved. The antibiotic used simultaneously with cortisone was penicillin, but as, according to Kass and Finland, the prognosis is so good from penicillin treatment alone, there was little to indicate whether the

effect of cortisone was adverse or beneficial. Again, an analysis of the treatment in all patients received at the Municipal Contagious Disease Hospital of Chicago by Lepper and Spies (1957) showed that there were 3 periods during this time, when hormones were restricted, when they were used rather freely, and finally almost eliminated except for fulminating meningococcal infection. Comparing the death rates in the 3 periods these workers were led to the conclusion that there was no effect, or possibly a deterioration in prognosis, in those patients treated during the era of free use of the hormones. These conclusions stimulated Lepper and Spies to make a controlled trial of the use of hydrocortisone in addition to appropriate antibiotics in bacterial meningitis. In this trial infections due to *H. influenzae*, pneumococci, and meningococci (when the latter were not particularly severe) were included. There was 1 death in each series, both due to pneumococcal infection. In other patients there was little evidence of a better symptomatic response in those treated with hydrocortisone and the abnormalities in the cerebrospinal fluid returned to normal more slowly than in controls treated with antibiotics alone (1957, *Lancet*, ii 888). In those patients with particularly severe meningococcal meningitis, all of whom received hydrocortisone intravenously as well as penicillin and streptomycin, there were 5 deaths and 2 survivors. In all those who died bilateral adrenal haemorrhage could be demonstrated.

In view of the mass of literature on the subject the few trials described here seem to provide a small counterblast to the enthusiasm of others. Yet conclusions from neither of the two controlled trials are in favour of the routine use of adrenocortical hormones in conjunction with antibiotics for the treatment of infections. At the end of their discussion of the subject Kass and Finland (1957) concluded that the value of corticosteroids in the management of infection was still uncertain except when there is manifest adrenal insufficiency. Even in these circumstances there are other vasopressor agents, including epinephrine, which are capable of preventing vascular collapse as adequately as is cortisone.

Gamma globulin

Of recent years attention has been drawn to the influence of 'host factors' in bodily defences. In this respect gamma globulin has been considered to play a part in enhancing the effect of antibiotic therapy. Following successful protective experiments undertaken by Fisher (1957) on mice infected with various strains of *Staph. aureus*, *Str. pyogenes*, *Proteus vulgaris*, and a strain of pseudomonas, Waisbren (1957) applied a combined therapy of gamma globulin and the appropriate antibiotic to a series of 46 stubborn cases of septic infection. In 6 of these a long period during which the administration of antibiotics had not induced signs of improvement preceded the giving of gamma globulin which was prescribed without changing the antibiotic or its dose. In these patients clinical response was immediate and was particularly successful in cases of chronic and acute osteomyelitis and staphylococcal pneumonia. Fig. 12 demonstrates the effect of the addition of gamma globulin to neomycin treatment in a stubborn case of staphylococcal pneumonia and lung abscess. It is of interest that though chronic osteomyelitis due to salmonellae responded satisfactorily, carriers of these organisms continued in the

carrier state without effect from the combined therapy. Although there was no evidence of a preliminary abnormally low level of gamma globulin in the blood of the successfully treated patients, yet response followed its addition and organisms were eliminated from cultures. This result was not thought likely to be due to the gamma globulin alone, for the condition of patients to whom only this agent was administered deteriorated until a suitable antibiotic was also given. The dose employed by Waisbren was 10 to 20 ml gamma globulin intramuscularly about 3 times a week.

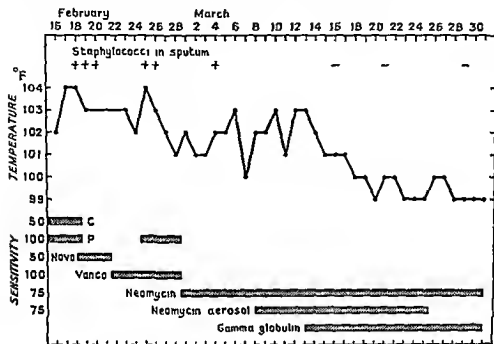


FIG. 12 Chart of a patient with staphylococcal pneumonia and lung abscesses successfully treated with gamma globulin and neomycin after chloramphenicol, vancomycin, penicillin, and neomycin alone had failed to cause amelioration of his disease.

C = chloramphenicol P = penicillin Novo = novobiocin Vanco = vancomycin

(From Waisbren *Antibiot. and Chemother.* 1957 7, 322.)

Other substances thought to influence the immune response of the host, such as properdin (Pillemer, Blum, Lepow, Ross, Todd, and Wardlaw, 1954) and other immune bodies have still to be tested in relation to their effect with antibiotics on bacterial infection.

Conclusion

In choosing an antibiotic or combination of antibiotics one is thus not only concerned with the disease for which it is to be given, but one is also dependent on some type of sensitivity test. Before deciding, however, it is also necessary to inquire into the history of the patient to determine whether the drug indicated by the sensitivity test has been given previously and how it has been tolerated. During treatment, serial weekly bacteriological examinations are advisable, in case the infection has been converted into one which is resistant and a change of antibiotic is required. When there appears to be resistance

to every available antibiotic singly, it is possible that a combination will be effective, but it is inadvisable to add more than one antibiotic at a time so that confusion does not occur. It is possible that at the present time the newer antibiotics may be used for infections which are resistant to those more commonly used, for example erythromycin, novobiocin, and vancomycin. If neomycin, bacitracin, or polymyxin is indicated, these drugs can be used in strictly controlled doses. When administration of a drug results in gastro intestinal upsets or toxic phenomena, a change of antibiotic is indicated. In this respect it is well to remember that the tetracyclines are, bacteriologically, largely interchangeable, but there are individual differences of activity against certain strains of bacteria. When the administration of streptomycin causes vestibular disturbances, dihydrostreptomycin may be given instead, but on no account should the dose of the latter drug be raised above 2 G daily, and preferably not above 1 G a day. For fungal infections, nystatin has shown itself to be useful either as a prophylactic or as a therapeutic agent. The value of other antibiotics in controlling fungal overgrowth has not yet been fully demonstrated in the clinic.

THE CHOICE OF AN ANTIBIOTIC IN DISEASES DUE TO SPECIFIC ORGANISMS

Certain infections can be recognized clinically and laboratory tests need only be made to confirm the diagnosis. In these illnesses there is little reason to delay treatment until the results of the tests are known.

Venereal diseases

Syphilis

Penicillin remains the first choice for the treatment of this disease in all its stages. With long acting preparations, such as procaine penicillin in oil with aluminum stearate, or benzathine penicillin, treatment can be given as a single injection of 2.4 to 6 million Units. When uncontrollable sensitization is known to be present the tetracyclines or chloramphenicol, or even erythromycin or carbomycin, can be used as second lines of defence (Olansky and Landman, 1950, Robinson and Robinson, 1951, and Romansky, 1953).

Gonorrhoea

Again procaine penicillin 600 000 or 1.2 million Units with aluminum monostearate given once is effective, but the tetracyclines and chloramphenicol, in a single dose of 3 G, are nearly as reliable. Erythromycin is also effective in divided doses of 200 mg each, amounting to a total of 800 mg in a day (Haight, 1956). With all these drugs however, serological tests to exclude syphilis need to be carried out for 4 months after treatment. When doubt exists however, streptomycin or dihydrostreptomycin (200 to 600 mg) can be safely used alone without fear of masking the more serious disease (Robinson, H. M., 1951 a).

Of late years there has been some doubt expressed as to the continuing

susceptibility of the gonococcus to penicillin owing to the number of cases which remain persistently resistant to treatment. Allowing for differences in technique, samples of strains tested from 1944 until 1956 do not lead one to imagine that lessened susceptibility is the cause of these resistant cases. The following figures are taken from Thayer, Field, Magnuson, and Garson (1957)

| | Year | No of strains | Range of sensitivity {U per ml} | Mean |
|-------------------------|------|---------------|------------------------------------|-------|
| Lankford (1945) | 1944 | 100 | 0.005-0.025 | 0.013 |
| Love and Finland (1955) | 1945 | 24 | 0.002-0.008 | 0.005 |
| | 1947 | 104 | 0.002-0.033 | 0.006 |
| | 1949 | 52 | 0.005-0.333 | 0.033 |
| | 1954 | 106 | 0.002-0.033 | 0.008 |
| Thayer (1956) | 1956 | 31 | 0.005-0.200 | 0.052 |

Thayer *et al* also point out that it took 48 hours or more for penicillin to kill all strains of gonococci although inhibition was manifest within 24. It is possible that preparations which do not maintain a suitable concentration of penicillin in the tissues for sufficiently long may be responsible for the failures of treatment with this antibiotic.

Granuloma inguinale

The tetracyclines and streptomycin are effective. The tetracyclines should be given in doses of 4 G daily for a minimum of 2 weeks, and streptomycin in maximum doses of 20 to 30 G given over 5 to 10 days. A third choice is chloramphenicol. Cases which become resistant to treatment with streptomycin, and those which relapse after treatment with the tetracyclines, do, however, occur. The latter sometimes respond to a second course of treatment with the tetracyclines (Greenblatt, Barfield, Dienst, and Zases, 1952, Knight and Fowler, 1956).

Lymphogranuloma venereum

The tetracyclines are effective also in this venereal disease but, in the few cases tested, relapses are frequent. From experimental work oxytetracycline appears to have been more effective than chlortetracycline. The doses given usually amounted to 1 G daily for 14 to 21 days (Banov, 1953, and Hurst, Peters and Melvin, 1950).

Chancroid

For this disease 1 G of streptomycin daily for 5 days is as effective as the tetracyclines given in doses of 0.25 G 4 times a day for 4 days, but streptomycin together with sulphadiazine has the advantage that it does not mask the presence of syphilis (Mendell, Foxworthy, and Wornas, 1954, Olansky and Landman, 1950 and Willcox, 1950). Penicillin is also useful particularly where facilities for diagnosis are not great and treatment for the disease is needed irrespective of the diagnosis (Willcox, 1954).

Rickettsial diseases

Chloramphenicol and the tetracyclines are all valuable in these diseases but it is well to remember that these antibiotics are not rickettsiocidal in spite of

the early dramatic results obtained with them. Relapses therefore are to be expected when a short course of treatment is given before the 7th day of disease, that is, before immune bodies have fully developed in the patient (Ley and Smadel, 1954). In a comparative study of the effect of chloramphenicol, chlortetracycline, and oxytetracycline among Chinese Nationalist troops in the Pescadores Islands, Prezyna, Teh Ling Chang, Tsu Lun Wang, Dougherty, and Bond (1954) found all 3 drugs about equally efficacious, but fever lasted for less time after chloramphenicol than after the tetracyclines, an average of 40 hours compared with 48 and 49 hours.

Diseases due to viruses

For diseases caused by the larger viruses of psittacosis, lymphogranuloma venereum, and trachoma chloramphenicol and the tetracyclines have proved themselves particularly valuable though they provide no guarantee against relapses: for example the case of French and Martin (1957). Effective treatment for the first 2 of these can be given by mouth and for trachoma by local application of an ointment. For other diseases usually attributed to viral infection, such as herpes simplex, mumps, measles, infectious mononucleosis, &c, there is no unequivocal evidence, experimental or clinical, that antibiotics have any specific effect. The undecided question whether primary atypical pneumonia is caused by a virus makes it difficult to assess the value of antibiotics in treating the disease. A number of comparative trials have been carried out (described by Finland, 1952) but in one series only had all the cases demonstrated the presence of the agglutinin reactions recognized to be specific for the disease. In this series no difference was noted between results in tetracycline treated patients and in those whose infection received only symptomatic treatment (Gallagher and Gallagher, 1952). Without the ability to isolate the specific virus treatment must necessarily be blind, and it is possible that the slight improvement in the results obtained with the tetracyclines compared with controls or penicillin treated cases might have been due to the prevention of superadded bacterial infection.

Bacterial diseases

Typhoid fever

In spite of the fact that chloramphenicol has similar inhibitory powers to the tetracyclines against the typhoid bacillus *in vitro*, it has undoubtedly proved the most effective agent in treating this disease. Although response to treatment may not be apparent for 3 or 4 days, *Salmonella typhi* disappear from the blood promptly and the patient recovers if intestinal haemorrhage or perforation does not occur. Relapses and the chronic carrier state are not prevented, although they occur less frequently if treatment is prolonged over 7 days or preferably to 14 days (Smadel, 1950 b). Carriers are usually treated with a combination of streptomycin and chloramphenicol, but there is no guarantee that this treatment in fact eradicates the carrier state. It is possible that synnematin B may prove more effective than chloramphenicol in eradicating *Salmonella typhi* from patients, but proof of this in clinical trials still awaits confirmation.

Other salmonellae infections

There is little prospect of available antibiotics controlling the infection in dysentery due to salmonellae. Chloramphenicol and streptomycin have been recommended, but their success in controlling the infection rather than in relieving the symptoms is variable. Two epidemics in nurslings were described by Marie, Seringe, Le Minor, and Elachbar (1951). In the first epidemic, 9 of the babies were treated with penicillin followed by streptomycin and one with streptomycin only. Five of these died. In the second epidemic, 8 infants were treated with antibiotics—streptomycin alone to 1 who died, streptomycin followed by chloramphenicol to 5, 2 of whom died, and chloramphenicol from the start to 2, both of whom recovered. Thus chloramphenicol brought the greatest relief, but there is no note as to whether these babies were prevented from becoming carriers. In urinary tract infections, however, streptomycin is the only drug, if given in doses which are related to the sensitivity of the organism, which will clear the urine of salmonellae within 24 hours (Garrod, personal communication).

Bacillary dysentery

When caused by shigellae this type of dysentery appears to be particularly susceptible to treatment by antibiotics as well as by the sulphonamides. Streptomycin has proved most effective when administered in doses of 60 to 80 mg per kg of body weight by mouth daily and 40 mg per kg by intramuscular injection (Chang and Su, 1951 and Forbes, 1953). Chloramphenicol and the tetracyclines, given in 3 doses amounting to 4 G over 24 hours, have cleared up many cases of sulphonamide resistant shigella infection (Garfinkel, Martin, Watt, Payne, Mason, and Hardy, 1953).

Whooping cough (Pertussis)

Although *Haemophilus pertussis* is inhibited *in vitro*, and in the embryonated egg, by concentrations of many antibiotics, which should easily be reached in the body, the results of treatment in patients have been equivocal. There were some enthusiastic and some guarded reports on the results of therapy with chloramphenicol or the tetracyclines but a collective and comparative study by the Medical Research Council (1953) produced little evidence that these drugs had much effect on pertussis if given after the first week of the disease. A similar conclusion was reached in the United States by Weinstein, Seltser, and Marrow (1951). There is, however, some evidence that the tetracyclines and chloramphenicol are valuable in controlling the complications of bronchopneumonia and encephalitis and in thus preventing deaths in the more severe forms of the disease (Minkenbof and van der Kooi, 1952). A daily dose amounting to 50 mg per kg of body weight, divided into 4 hourly or 6 hourly amounts, is given for a week to 10 days.

Brucellosis

It is not difficult to control the acute manifestations of brucellosis with a number of different antibiotics. It is very much more difficult to rid the patient of the infection. For this reason 2 drugs have been used together in the hope that one would act on bacteria insusceptible to the other or in sites where the other was ineffective. Streptomycin in a dose of 2 G a day with

sulphadiazine in a dose of 6 G daily given at 4 hourly intervals has been used with good effect by Spink, Hall, Shaffer, and Brande (1948 and 1949). Later, however, a combination of streptomycin with chlortetracycline was found to be more efficient both in the rapidity of its action and the effectiveness with which it reduced the bacterial population in blood and tissues (Werner and Knight, 1950). This finding was confirmed clinically by Herrell and Barber (1952), Janbon and Bertrand (1949), Harris (1953), Magill, Killough, and Said (1954), and Spink (1955). The doses recommended were up to 3 G of chlortetracycline by mouth daily¹ and 1 G of streptomycin or dihydrostreptomycin injected intramuscularly in 2 doses a day, for 12 to 14 days. When cases were already chronic, the addition of brucella antigen to the therapy was said to be useful. In acute or particularly toxic cases, cortisone in doses of 300 mg gradually reduced to 100 mg over 4 to 5 days has been claimed to be of use.

Tularaemia

Streptomycin has proved particularly successful in the treatment of tularaemia. A large daily dose is not required. One to 1.6 G daily, injected at 4 or 6 hourly intervals and continued for 7 days, has been found sufficient to control the infection so long as there is no abscess formation in the lymph nodes. Recurrences may occur later, but since no *Past tularensis* resistant to streptomycin has yet been isolated, a repetition of the course of treatment is advised (Foshay, 1947, and Rosenthal, 1951). Chloramphenicol and chlortetracycline were found to be as effective as streptomycin in the early febrile state of the illness (de Lavergne, Pierquin, Helluy, and Dormer, 1950), but these drugs have not been so extensively tested.

Plague

Streptomycin has been shown to have a very definite beneficial effect on this disease. Sokhey, Wagle, and Habbu (1953), comparing the mortality from bubonic plague in certain districts in India following treatment with various sulphonamides and treatment with streptomycin, showed that although some reduction in mortality followed the introduction of each new sulphonamide, treatment with streptomycin resulted in an even lower death rate (4.2 per cent in 148 cases). Comparisons with other antibiotics are not available, but it is worthy of note that one patient with pneumonic involvement recovered after being treated with chlortetracycline and penicillin (Public Health Reports (Washington), 1949).

Bartonellosis

Little is known of the effects of different antibiotics on this disease, which is mainly confined to Peru. Krumdieck (1949) studied the effect of antibiotic treatment in 50 cases. This author found that penicillin, given in a dose of 200,000 Units daily, caused the bartonella to disappear from the blood in the pre-eruptive phase, but had little effect on the eruption. A similar result was seen in 1 case treated with streptomycin. Chloramphenicol, however, given by mouth in doses of 50 mg per kg of body weight per day, did have some effect on the eruption. The eruption recurred, however, after treatment had been continued for 3 days only.

¹ Later Spink (1956) recommended tetracycline 0.5 G by mouth 4 times a day for 3 weeks.

Anthrax

Tests of the sensitivity of *Bacillus anthracis* to various antibiotics have been carried out by several workers in different parts of the world. These have not necessarily used the same method of testing but all agree in finding no antibiotic more active than penicillin against the most sensitive strains. The figures of sensitivity are given below. The 18 strains of *Bacillus anthracis* were collected by Garrod (1952) from different parts of Great Britain as well as from the National Collection of Type Cultures. He used a method of doubling dilutions of the antibiotics in solid media.

| No. of strains | Penicillin* | Units or μg per ml inhibiting growth | | | |
|----------------|-------------|---|--------------------------------|------------------------------|------------------------------|
| | | <i>Strepto- mycin</i> | <i>Chlortetra- cycline</i> | <i>Chloram- phenicol</i> | <i>Oxytetra- cycline</i> |
| 6 | < 0.1-5.0 | 3.12-12.5 | < 0.1-1.0 | 3.12-12.5 | 0.1 |
| 6 | < 5.0 | < 5.0 | 5.0 | > 5.0 | |
| 18 | 0.009-0.037 | 0.6-1.25 | 0.075-0.15 | 2.5-5.0 | |
| 29 | 0.1-5.0 | 3.12-25 | 0.1-1.0 | | 0.1-1.0 |

* Units per ml

Figures obtained from the data of Boger (1953), Garrod (1952), Gold and Boger (1951), and Ruiz Sánchez, de Leon, Villegas, and Novelo (1949).

All 37 strains found by Gold and Boger (1951) and Boger (1953) were found to be resistant to 25 μg per ml of polymyxin and to be inhibited by 3.12 to 25 μg per ml of neomycin, but all tests agree in finding no antibiotic more active than penicillin against the most sensitive strains. In Garrod's series the strains requiring the highest concentration of penicillin were still more sensitive to this drug than to any of the other antibiotics tested, the range of inhibitory concentrations being 0.009 to 0.037 Unit per ml. However, this was not the case in the series described by Gold and Boger (1951) or Boger (1953). The tetracyclines and chloramphenicol, as might be expected from the figures given above, had been successful in clinical trials. Gold and Boger (1951) treated 8 cases with chlortetracycline, chloramphenicol, or oxytetracycline, all with equal success. The local oedema and erythema were controlled within 24 to 48 hours and *Bacillus anthracis* disappeared from the lesions. In 1953 Boger tested a further 21 strains and found that the clinical results correlated well with the sensitivity tests. Ganter (1953) treated 14 cases and considered penicillin injected in doses of 250,000 Units or more daily for 1 week and infiltrated locally at the site of the lesion to be superior to other antibiotics. However, this author used sulphonamides as well in complicated cases. By 1955 Gold had treated 40 cases with antibiotics, including erythromycin. Chloramphenicol had been abandoned owing to the reports of its depressive effect on the haemopoietic system, but the tetracyclines and erythromycin were found to work equally well. These were preferred to penicillin because the serious complications of anthrax were no longer to be feared and these antibiotics could be administered to patients without admitting them to hospital. With the advent of phenoxymethylpenicillin, since therapeutic blood levels can be obtained by its oral administration, the main objection to prescribing this antibiotic has disappeared. In the absence of any other contraindications penicillin must still be regarded as the first choice for anthrax. It has had the longest trial and remains particularly effective in this condition.

Actinomycosis

Comparative studies of the sensitivity *in vitro* of strains of actinomyces isolated from lesions in man have been made by several investigators. Strauss, Khigman, and Pillsbury (1951) compared the activity of 4 antibiotics against 3 strains of *Actinomyces bovis*. According to their observations, made under conditions which were unsatisfactory, at least for penicillin, chloramphenicol should be the most active antihototc therapeutically. However, only a small number of cases have been treated by chloramphenicol. The response with chlortetracycline, however, seems to have been uniformly successful. Garrod (1952) tested 12 strains of *A. israeli* from different sources, 3 isolated from patients admitted to St Bartholomew's Hospital, London, by a serial dilution method in specially prepared broth. The effects of 5 different antibiotics were tested. Eleven strains of the fungus were inhibited by penicillin in lower concentrations than oxytetracycline, chlortetracycline, chloramphenicol, or streptomycin. Eighteen other strains subsequently examined by the same author were still inhibited by concentrations of penicillin of 0.25 Unit per ml or less. It is possible that the conditions of the experiment were not favourable for at least 2 of the other antibiotics: the long incubation period (5 days) would allow deterioration of chlortetracycline to occur and the presence of the CO₂ required for the anaerobic cultivation would have lowered the pH of the medium, making it unfavourable for the action of streptomycin. A later comparative series of sensitivity tests was carried out by Suter and Vaughan (1955) and Suter (1957). The earlier study included 11 antibiotics but not penicillin. Against 3 strains of *Actinomyces bovis*, erythromycin appeared to be the most active. Moreover, when exposed for long periods to erythromycin, penicillin, or carbomycin, strains gave no sign of acquiring resistance. Resistance to the tetracyclines and chloramphenicol in the same time (16 weeks) had increased 12 fold.

Clinical tests in the last 5 years have not yet decided which antibiotic is the most active. Kelly (1951) successfully treated a patient suffering from abdominal actinomycosis initially with penicillin, sulphadiazine, streptomycin and incision of abscesses, and later with chloramphenicol and chlortetracycline. Sangster (1952) treated a case of actinomycosis of the jaw with chlortetracycline, potassium iodide, and penicillin. A rapid response was obtained. Pannekoek (1954), however, described a patient who died from hepatic actinomycosis following septicaemia due to *E. coli*, despite treatment with penicillin and streptomycin. These cases of actinomycosis were presumably caused by *Actinomyces israeli*. The above work would seem to indicate that for actinomycosis caused by *Actinomyces israeli*, penicillin should be the first choice, whatever antibiotics might be used subsequently. No-cardia have not been found susceptible to penicillin, but according to Strauss *et al* (1951) they are inhibited by therapeutic concentrations of both chlortetracycline and sulphadiazine, and according to Banerjee, Sen, and Nandi (1954) by streptomycin or chlortetracycline. The clinical data only show the effectiveness of sulphadiazine after unsuccessful administration of penicillin and streptomycin, and sometimes after chlortetracycline had also met with no success in clearing up the condition (Bernstein, Cook, Plotnick, and Tenczar, 1952, Connor, Ferguson, Sealy, and Conant, 1951, and Mogabgab and Floyd, 1951).

Tuberculosis

Whether or not antibiotics will continue to be the main standby in the therapy of tuberculosis remains in doubt. At the present time, however, streptomycin together with another drug (isoniazid or PAS) appears to be the indicated therapy in most cases. One gramme of streptomycin daily or every 2 or 3 days, together with isoniazid or PAS daily, is usually a big enough dose to produce a therapeutic effect if the treatment is continued for long enough. Therapy for a year or longer has sometimes markedly improved gross anatomical changes which it had earlier been thought could only be relieved by surgical means.

Leprosy

No antibiotic has been found to be curative in this disease but streptomycin combined with sulphone therapy has been useful in reducing lesions of the skin and mucous membrane. Ulcers of the mouth have healed within 6 weeks with this therapy. Chlortetracycline, administered in doses of 1 to 1.5 G daily for a year, has also healed lesions of the skin and mucous membrane. This prolonged treatment was generally accompanied by gastric intolerance at some stage (Johansen and Erickson, 1950).

Protozoal and other infections

Amoebiasis

A number of effective anti amoebic drugs were in use before the advent of antibiotics with a similar action. The problem has therefore been to find antibiotics which were more effective than other drugs or were valuable as adjuvants. By 1950 McVay, Laird, and Sprunt had made a trial of the effect of chlortetracycline in 37 patients, using a dose of 0.5 G 4 times a day. The patients responded very well, both trophozoite and cystic forms disappeared. Administration of oxytetracycline in doses of 2 G by mouth daily in divided doses for 10 days met with equal success in the hands of Most and van Assen delft (1951), only 1 case relapsed. Bacitracin, given in doses of 80,000 Units daily by mouth for 10 days, was also found effective in relieving the symptoms and removing *E. histolytica* from the stools (Most *et al.*, 1950 b). At about this time Bradin and Hansen (1950) drew attention to the fact that amoebae required the presence of bacteria in the medium for growth. These authors also showed that, in evaluating a therapeutic agent, tests under both aerobic and anaerobic conditions were necessary. Streptomycin, chlortetracycline, and bacitracin were found to be effective only in an indirect way, that is, by eliminating the bacteria from the cultures of amoebae, and thus inhibiting the protozoa. On the other hand, emetine destroyed *E. histolytica* directly. W. R. Jones (1950) carried out experimental work which confirmed the above findings. None the less chlortetracycline inhibited the growth of 1 strain of *E. histolytica* at concentrations of 1/90,000 and another strain at concentrations of 1/30,000. Although chloramphenicol behaved in the same way as the 2 tetracyclines, penicillin and streptomycin were less active. At the same time experimental trials in rats carried out by Seneca and Henderson (1950) showed that although penicillin and phthalylsulphathiazole had no action *in vitro* against *E. histolytica*, these drugs administered prophylactically for

2 days reduced the number of animals which became significantly infected. Further confirmation of the value of chlortetracycline in experimental and clinical work was given by Watt and Van de Grift (1950). These authors determined the dilution of each antibiotic at which the amoebae died and the concomitant bacteria survived. The results, expressed in mg per ml, were as follows

| | |
|-------------------|-----------|
| Chlortetracycline | 0.22-0.25 |
| Polymyxin B | 0.18-0.22 |
| " D | 2.0-2.5 |
| " E | 0.5-0.55 |
| Circulin | 2.25-2.5 |

When 2 strains of amoebae were treated with a dilution of the drug which just allowed the amoebae to survive, a slight increase in resistance was observed to chlortetracycline. Nevertheless, 6 patients given 7 to 10 G of chlortetracycline over 4 to 5 days no longer showed *E. histolytica* in their stools at the end of 3 days and remained free of amoebae for the following 20 to 25 days. On the other hand, Felsenfeld *et al.* (1951) found that neomycin and bacitracin in combination were more active than penicillin, streptomycin, chloramphenicol, or the tetracyclines. In a clinical trial 48 patients received 1,000 to 40,000 Units of neomycin and 250 to 500 Units of bacitracin per kg of body weight per day by mouth for 10 to 14 days. This course was repeated in a week's time if necessary. A good response was obtained in all cases and there were no side effects during treatment. After 3 to 6 months no relapses had occurred in 41 of the patients. Another combination recommended by Seneca (1956) was that of tetracycline, nystatin, and novobiocin. Although chlortetracycline seemed to be an effective anti-amoebic agent, the number of cases in which relapses occurred was sufficient to warrant further inquiry into which was the most satisfactory drug. Sodeman (1951) quoted the cure rates for bacitracin (90,000 Units by mouth daily for 10 days) as 66 per cent and for chlortetracycline (1 to 2 G daily for 10 days) as 79 per cent, after 1 year (Most, Muller, and Grossman, 1950a). Eldon Dew, Armstrong, and Wilmot (1952) reported that chlortetracycline and tetracycline relieved the acute dysentery in the great majority of patients but that there were many relapses following the cessation of treatment. This was also apparent in the observations of Tohie, Most, Reardon, and Bozicevich (1951). These authors subjected carriers of *E. histolytica* to treatment with chlortetracycline or bacitracin or oxytetracycline. These carriers were in 4 separate institutions and were strictly isolated. The results, therefore, should not have been vitiated by the occurrence of reinfection. These results were as follows

| | Treatment | | |
|--|-------------------|------------|-----------------|
| | Chlortetracycline | Bacitracin | Oxytetracycline |
| | % | % | % |
| No <i>E. histolytica</i> found in stools after 2 weeks | 100 | 59 | 100 |
| " " " 2 to 5 months | 60 | 28 | 100 |
| " " " 6 months | | | 100 |

From the figures it would seem that oxytetracycline was an improvement on chlortetracycline. These findings led some investigators to conclude that the effect of all the antibiotics so far tried was due to the eradication of the bacteria on which the growth of the amoebae depended (Harvill, 1952, and

Spingarn and Edelman, 1952) Various combinations of antibiotics were then tried together with amoebicidal agents This therapy was nearly always immediately successful but did not eliminate relapses Similar results were obtained by Martin, Garfinkel, Brooke, Weinstein, and Frye (1953), Thompson and Reinertson (1951), and Zavala and Hamilton (1952) The latter authors used chlortetracycline or oxytetracycline and Goldenberg, Shlaes, and Mintzer (1952) used bacitracin

One group of investigators attempted to compare the effects of the tetracyclines alone (Weiser, Spiotta, and Elman, 1953), but fell back on emetine after 1 or 2 courses of oxytetracycline had failed to prevent relapses The clinical effect of different antibiotics was compared once more by McHardy and Frye (1954), who tested fumagillin as well The results were as follows

| <i>Antibiotic</i> | <i>No. of cases surveyed</i> | <i>Percentage of failures or recurrences</i> |
|-------------------|------------------------------|--|
| Chlortetracycline | 697 | 16.8 |
| Oxytetracycline | 435 | 8.5 |
| Bacitracin | 205 | 31.2 |
| Chloramphenicol | 72 | 73.6 |
| Neomycin | 22 | 66.3 |
| Fumagillin | 119 | 14.0 |

This list still favours oxytetracycline even though treatment was continued long enough for intestinal bacteria to have become resistant to this and the other antibiotics When a comparison was made between oxytetracycline and the amoebicide fumagillin (Anderson, 1955) it was again found that results were in favour of oxytetracycline The figures were as follows

| <i>Treatment</i> | <i>Total</i> | <i>No. cleared of E. histolytica</i> |
|--|--------------|--------------------------------------|
| Oxytetracycline (0.25 G 3 times daily for 10 days) | 18 | 14 |
| Fumagillin (5-10 mg daily) | 20 | 13 |

Anderson (1955) pointed out that results differed according to the locality in which the patient lived For example, natives of South Africa, or of Indonesia or Burma have infections which are complicated by many other diseases so that any drug used should be not only anti amoebic but anti-protozoal in general Anderson used erythromycin in the treatment of children with amoebiasis In a series of 52 cases, fumagillin alone cleared only half the patients, while erythromycin cleared approximately 93 per cent A combination of the 2 drugs was no better than erythromycin alone Balamuth (1955), however, found erythromycin and the tetracyclines to have only moderate amoebicidal activity, and he was unable to confirm the clinical effectiveness of chlortetracycline or oxytetracycline Wilmot (1956), working among the African population of Durban, South Africa, compared the immediate results of treatment with the 3 tetracyclines One gramme was given daily for 15 days Twenty seven days after the beginning of treatment, there was little difference in the results of the three groups The cure rate in 136 cases at this time was in the neighbourhood of 94 per cent The position is thus at present still ill defined It should, however, be remembered that amoebae require the presence of bacteria for growth even *in vivo* The necessity of controlling both infections in order to cure amoebiasis is borne out by the experiments of Phillips, Wolfe, Rees, Gordon, Wright, and Reyniers (1955),

who found that when guinea pigs reared under sterile conditions were infected intracaecally with amoebae, the amoebae did not grow unless *A. aerogenes* or *E. coli* were introduced into the caecum at the same time. If the same is true in man, it would seem that the object of treatment should be first to control the various bacteria in the gut lumen and then to apply an amoebicidal drug capable of penetrating the tissues, since histologically the amoebae are found to have invaded the gut often as far as the muscularis mucosae. At present, neomycin and bacitracin, erythromycin, or 1 of the tetracyclines, can be administered, followed within a few days by fumagillin, or some other amoebicide such as carbarsone milibis, or a quinolino derivative. These treatments, similar to those found most effective by Loughlin and Mullin (1936) or McHardy, Welch, Browne, Blum, and McHardy (1955), are also recommended by Seneca (1955) and, according to Thompson (1955), require the lowest doses of drugs compatible with recovery in man.

Yaws and pinta

These spirochactal diseases have both been treated effectively with penicillin but there are also good reports of the use of chloramphenicol and the tetracyclines in yaws. A dose of 1 G. of one of these latter antibiotics daily is given for adults, and 0.25 to 0.5 G. daily for children. However, for ease of administration, a '2 shot course' of treatment with penicillin preparations far surpasses the other drugs, since children sometimes have difficulty in swallowing the tablets (Hill, 1953).

Leptospirosis

Although penicillin has been used widely in this country, there has been no reduction in the death rate from leptospirosis. This death rate was 15 per cent. before the introduction of penicillin and has remained at this level (Broom 1951). However, since the susceptibility of strains varies considerably, it is possible that the dosage used was not high enough. In a series of 70 cases due to various leptospira treated in the United States, penicillin, streptomycin, chloramphenicol, or the tetracyclines were employed, and there were only 2 deaths (Hall, Hightower, Rivera, Byrne, Smadel, and Woodward, 1951). This reduced death rate seems to indicate that antibiotics had some effect. Except for penicillin, where the dose was about 500,000 Units or more daily for 5 to 20 days, the remaining antibiotics were administered usually in a high dose initially and then in doses of 2 to 3 G. daily for not longer than 14 days. There is some indication that erythromycin, carbomycin, and oleandomycin may destroy leptospira at high concentrations (Cook and Thompson, 1957).

Fungal infections

These have come into greater prominence since the introduction of antibiotics. For fungal infections nystatin seems now to have been well tried. It can be applied locally in concentrations of 5,000 to 100,000 Units per ml. or G., or be given by mouth in doses of 500,000 Units 6 hourly to adults and 50,000 to 100,000 Units 4 times a day to infants and young children (Huang, Kendall, Lamberti, and High, 1956).

THE CHOICE OF AN ANTIBIOTIC IN DISEASES CONSIDERED BY SYSTEMS

Wherever the infection may be, it is most important to find out what the infecting organism is. Certain areas are, of course, the site of election of certain bacteria, for example, the skin, subcutaneous tissues, and bones are more commonly invaded by staphylococci than other bacteria, the urinary tract is most frequently invaded by Gram negative bacteria, and the heart valves by *Str viridans*. Although one can often make a good guess as to the causal organism, experience in recent years has shown that the susceptibility of a particular strain to the various antibiotics must be tested. Sensitivity tests with all their limitations are in the great majority of instances a better and quicker guide than any clinical assessment can be. Boils, for example, are commonly due to the staphylococcus, but unfortunately resistant strains of this organism are increasingly prevalent. A direct culture on to a plate containing disks impregnated with the various antibiotics should make it rapidly known which antibiotic, if any, is indicated in the treatment. A list of organisms and the antibiotics usually found active against them in clinical trials is added below to guide the clinician in his first choice of therapy before the results of sensitivity tests are known to him.

Staph aureus

| | | |
|-----------------|---|--|
| Erythromycin | } | At the present moment these are the most reliable agents for patients in hospital |
| Chloramphenicol | | |
| Vancomycin | | |
| Penicillin | } | For the patients living at home |
| Tetracyclines | | |
| Novobiocin | } | Both should be used for short periods as they induce resistance in the staphylococci while under treatment |
| Spiramycin | | |
| Bacitracin | } | By mouth only—for the gastro intestinal canal |
| and | | |
| Neomycin | | |

Str pyogenes

Penicillin
Tetracyclines
Erythromycin
Spiramycin
Novobiocin
Vancomycin

Pneumococcus

Penicillin
Tetracyclines
Chloramphenicol
Erythromycin
Vancomycin

Enterococci

Penicillin and Streptomycin

Streptomycin and Chloramphenicol, and/or the Tetracyclines

H. influenzae

Chloramphenicol

Streptomycin

Polymyxin

Erythromycin

E. coli

Tetracyclines

Chloramphenicol

Framycetin

Neomycin

Polymyxin E

Cycloserine with another antibiotic

Proteus

Novobiocin

Penicillin and Chloramphenicol in high doses

Ps. aeruginosa

Polymyxin E

Neomycin

Cycloserine with another antibiotic

***Bact. friedlander* (or *Klebs. pneumoniae*)**

Streptomycin and Tetracyclines

Fungi

Nystatin (clinical trials of other anti fungal agents have not yet fully substantiated the claims made for them)

In using these antibiotics dosage schemes are usually required, and those given below, although not necessarily ideal in all circumstances, have been extensively tried.

Penicillin Crystalline salt any dose by mouth or, more frequently, by intramuscular injection, from 500,000 Units 8 hourly upwards

If combined as a slow acting salt, from 300,000 Units 12 hourly upwards

Phenoxyethylpenicillin 200,000 to 500,000 Units by mouth in tablet, capsule, or oral suspension, the dose varying according to whether the susceptibility of the invading organism is below or above 0.02 Unit per ml

Streptomycin 1 G daily, or, if given for not more than 3 weeks, 2 to 2.5 G daily in 6 hourly injections according to the patient's reaction

Chloramphenicol 50 to 100 mg per kg of body-weight per day by mouth (for precautions see Vol III, p 15, in special section on Chloramphenicol).

Tetracyclines 0.25 to 0.5 G 6 hourly

Bacitracin 40,000 to 80,000 Units daily by intramuscular injection, not more than 100,000 Units per 24 hours

- Neomycin For parenteral administration in adults—0.25 G 6 hourly, not more than 0.5 G 6 hourly
- Polymyxin E 2.5 mg per kg of body weight per day by intramuscular injection, not more than 4.8 mg per kg per day
- Cycloserine 0.5 to 1 G daily by mouth in at least 2 doses
- Spiramycin 3 G daily in 3 to 4 doses by mouth, 75 to 100 mg per kg per 24 hours to children
- Oleandomycin { 500 mg by mouth 4 hourly, or 40 mg per kg of body-weight per day to children
- Novobiocin 5 to 10 mg per kg of body weight every 6 to 8 hours Or 2 G daily in divided doses to adults
- Nystatin 100,000 Units per ml or G for local application 2 to 4 million Units daily by mouth to adults, in divided doses
- Vancomycin 0.5 to 1 G by intravenous infusion every 6 to 12 hours
- Puromycin 1 to 2.25 G by mouth daily for 7 to 10 days

The following drugs have been omitted from the list of antibiotics recommended in clinical practice oleandomycin, spiramycin, and carhomycin. Each of which has similar activity to erythromycin with the disadvantage that staphylococci show cross resistance between them all. Where erythromycin is not available one of these other antibiotics can satisfactorily take its place.

- Staphylomycin }
 Ristocetin } Owing to the small number of published clinical trials these
 Puromycin } antibiotics cannot be recommended with certainty

Bacterial Endocarditis

The great majority of cases of bacterial endocarditis are due to *St. viridans* and this organism rarely fails to respond to penicillin (Christie, 1953, Keefer, 1953). However, since the body defences are poor when the streptococci are in this peculiar anatomical situation, bactericidal rather than bacteriostatic concentrations of penicillin are required, that is 4 to 5 times the concentration required to inhibit growth of the organism. Although some patients have recovered by oral administration of phenoxymethylpenicillin, such concentrations at the present moment are more readily ensured by repeated parenteral injections or by continuous infusions rather than by oral administration. Levinson, Griffith, and Pearson (1950) administered as much as 20 million Units daily even when the causal streptococci were inhibited by 25 Units per ml. That penicillin can penetrate the fibrin layer covering the valves in which the streptococci are enmeshed has been well shown by Nathanson and Liebbold (1950). These authors placed pieces of vegetations from a patient who had died after 3 days' treatment with penicillin, on the surface of seeded agar plates and demonstrated that a zone of inhibition was produced round each fragment. During the past 5 years various antibiotics have been employed, sometimes with success, but seldom before penicillin was tried first. It is most important in this condition to isolate the organism and test its sensitivity, preferably by a serial dilution method. Good agreement between sensitivity tests *in vitro* and clinical response to antibiotic therapy was found by Froment, Monnet, Gonn, Viallet, and Traeger (1950).

A marked change in the incidence of this disease has been noted by Littmann and Schaaf (1950), whose survey of 97 cases treated between 1944 and 1949 showed that the incidence of the disease had fallen by nearly half during the second part of this period. This was ascribed to the prophylaxis resulting from the treatment of relatively trivial complaints. These authors also noted that in those patients who did acquire the disease, the invading organisms had become steadily more resistant. This would seem to be the conclusion drawn by Bunn and Cook (1954), who used a routine dose of 12 million instead of 1 million Units daily until the sensitivity tests were known. An instance was cited by Loewe *et al* (1953) in which the *Str viridans* was inhibited only by 12 Units of penicillin per ml, but it was eventually controlled with probenecid, dihydrostreptomycin, and bacitracin. There are, however, organisms besides *Str viridans* which occasionally have to be dealt with. These include streptococci belonging to Lancefield group D, or enterococci which may be described as *Str faecalis* or *Str zymogenes*, staphylococci, brucellae, *H influenzae*, salmonellae, *Ps pyocyanea*, and others. Each of these infections requires its own treatment and it is possible that combinations of different antibiotics have been of value in those cases which were resistant to penicillin. Examples of such cases have been given above, but there are others in which, although a succession or combination of antibiotics have resulted in cure, there is no unequivocal evidence that the final combination had not other antibiotics earlier eliminated some of the bacteria. The infections of the heart valves which are now to be discussed have been treated with success in some instances, but a warning is given that early successes provided no guarantee that similar therapy will subsequently be effective. Whether this is so or not depends very much on the bactericidal effect of the antibiotic or combination of antibiotics on the particular organism concerned and on the power of the antibiotics to penetrate the fibrin covering the infected heart valves.

Enterococcal infection. Although both penicillin, with or without probenecid, and streptomycin have been used singly to cure an occasional case of this infection, a combination of the 2 drugs has been by far the most successful form of treatment (for example, Cates, Christie, and Garrod, 1951, Balme and Dormer, 1954, and Tompsett, 1953). Geraci and Martin (1954a and b), reviewing the 33 cases of enterococcal infection which they had treated during the previous 10 years, also found penicillin and dihydrostreptomycin to be the most effective combination. When erythromycin or bacitracin were added the results were no better. Nevertheless, in a relatively insensitive infection, it is sometimes necessary to raise the dose of streptomycin above 2 G daily for more than 3 weeks, this entails the risk of toxicity, and there have been occasions when raising each separate dose of penicillin to a million Units or more has still not been enough to effect a cure. It is in these instances that one may have to look for other treatment. The following drugs have been used successfully in isolated cases.

Chlortetracycline was effective in curing 1 of 2 patients described by Harvey, Mirick, and Schaub (1949). In another case, when chlortetracycline, oxytetracycline, and chloramphenicol singly had been ineffectual, all 3 antibiotics given for 6 weeks in relatively small doses, together with *Gantrisin*, eventually cleared the blood stream of enterococci (Friedberg, 1952). In

other cases cure was obtained by giving penicillin together with chlortetracycline (Friedberg, 1952, and Beattie, 1954) Chlortetracycline together with oxytetracycline in doses of 0.5 G given by mouth 6 hourly were successful in 1 case in which penicillin and streptomycin had failed (Rubenstein and Austin, 1952) Penicillin and bacitracin cured another patient, the dose of bacitracin given did not exceed 18,000 Units daily, but 4 million Units of penicillin were administered over the 24 hours One case in which erythromycin effected a cure after penicillin, streptomycin, and oxytetracycline had been unsuccessful was described by Lambert, Lamalle, and Lieveins (1955), while another, given 600 mg 4 hourly of the newer antibiotic, failed to be rid of her infection till she received 'massive' doses of penicillin and streptomycin (Romansky, Nasou, Davis, and Ritts, 1956) Novobiocin was considered responsible for the recovery of 1 patient treated by Pearson *et al* (1956) and ristocetin for that of another by Romansky *et al* (1957)

Staphylococcal infection In spite of the increasing number of penicillin resistant strains of staphylococci isolated from patients, Tompsett (1953) was able to cure some cases with resistant organisms by repeated doses of 100 million Units of penicillin, together with streptomycin or dihydrostreptomycin When this failed the addition of a tetracycline, or even bacitracin, was sometimes effective Penicillin together with bacitracin were found effective in a case described by Friedberg and Bader (1951) and penicillin with chlortetracycline in a second case described by Miller, Hansen, and Pollock (1954) Oxytetracycline alone cured a case in which the staphylococcus was inhibited by 2 μ g of the antibiotic per ml Streptomycin alone resulted in cure in some patients treated by Hunter (1947) and by Terry (1948) and vancomycin cured 1 case treated by Geraci *et al* (1957) Streptomycin together with oxytetracycline cured a patient treated by Miller *et al* (1954) Chlortetracycline alone in large doses, 1.5 G 4 hourly initially and later 0.5 G 4 hourly for 6 weeks cured an infection due to a *Staph albus* which was very sensitive to the drug (Hughes, 1951) Oxytetracycline together with streptomycin in doses of 3.4 G daily cured another case in which the organism was inhibited by the 2 drugs together, each in a concentration of 10 μ g per ml A concentration of 20 μ g per ml of each drug was required for inhibition if they were used singly A child described by Johnson and Hurst (1954) was cured by erythromycin together with oxytetracycline and streptomycin given intramuscularly, after unsuccessful treatment first with 60 million Units of penicillin daily by intravenous injection together with streptomycin and then with erythromycin together with streptomycin A case of a double infection with a staphylococcus and a *Str viridans* described by Ahern and Kirby (1952) recovered after 600,000 Units of procaine penicillin had been administered 3 times a day by intramuscular injection together with 1 G of chloramphenicol 6 hourly Miller *et al* (1954) described a cure in a case of staphylococcal infection after 2 G of chloramphenicol had been given 4 hourly

The fallacy of accepting the most recently tried antibiotic as being most effective is illustrated by the case described by Phillips, Romansky, and Nasou (1955) This patient developed a staphylococcal endocarditis following treatment by cortisone of an exfoliative dermatitis due to penicillin The history thus excluded the latter antibiotic from treatment, but sensitivity of the bacterium to erythromycin, chloramphenicol, and bacitracin was

demonstrated Erythromycin was naturally given as the antibiotic of choice in doses of 400 mg 6 hourly, but this had little clinical effect over 3 weeks. By this time the staphylococcus had become resistant to erythromycin. The addition of streptomycin, and then of bacitracin, to the treatment did produce a dramatic but only a transient response to the temperature curve, and did not otherwise alter the course of the disease. Blood cultures remained positive in spite of the fact that chloramphenicol was added to the treatment. At post mortem, a ruptured aortic cusp was found and there were multiple abscesses in the throat, liver, spleen, and kidneys.

With a rapidly bactericidal drug such as neomycin, it was found possible to cure a patient with the relatively low dose of 0.25 G injected 6 hourly for 1 week, followed by half this dose for the following 4 weeks. This case had previously been treated unsuccessfully with penicillin, streptomycin, chlorotetracycline, and oxytetracycline. The only untoward effect of treatment was a buzzing in the ears (Reed and Wellman, 1953). This antibiotic was again curative in a case in which massive doses of penicillin together with probenecid and streptomycin had failed (Beckwith, Goyette, Spicer, and Grahner, 1954).

Despite the difficulties in the treatment of this condition, Fisher, Wagner, and Ross (1955), in surveying the 38 cases of staphylococcal endocarditis treated at the Johns Hopkins Hospital over the 20 years between 1933 and 1953, found that while 1 case only had survived in the first 10 years before antibiotic therapy was introduced, the number of survivals had increased during the second 10 years to 1 out of 3 of those treated with penicillin and 7 out of 13 of those treated when other antibiotics also became available. What is of particular interest is the claim made by these authors that recovery and the sensitivity of the staphylococcus to the antibiotics bore no definite relationship, and that it was not possible to forecast the inhibitory action of 2 antibiotics administered since the concentrations *in vivo* were perpetually changing.

This claim might be countered by the account given of the successful eventual treatment of a case by Jawetz *et al* (1955). When the staphylococcus, which during 7 months proved refractory to so many antibiotics, including penicillin, was tested by their method and proved to be susceptible to combinations of streptomycin with oxytetracycline or with bacitracin, treatment with the former combination sterilized the blood and with the latter brought about full recovery.

Infection due to Actinomyces visus This very rare condition was encountered once by Stokes, Gray, and Stokes (1951) and was treated with chloramphenicol in doses of 1 G initially and then 0.5 G 4 hourly for 28 days. Emboli which had been a feature of the condition, ceased after 1 week's treatment.

Corynebacterium infection Instances have been given of recovery from a diphtheroid infection following treatment with penicillin and bacitracin (Zendel and Lubart, 1952) and following treatment with streptomycin and penicillin by McCoy and Meyer (1948).

Pneumococcal infection Although this organism is particularly sensitive to antibiotics, by the time it invades the heart valves the patient is usually in poor condition owing to a pre-existing and uncontrolled pneumonia. Recoveries have seldom been recorded. Providing no risk of sensitization is apparent the antibiotic of choice is penicillin.

Bacteroides infection An anaerobic bacteroides isolated from a case of pericarditis was found by Rantz (1951) to be sensitive to 1 μg per ml of oxytetracycline. Treatment was accordingly given in a dose of 3 G daily for 6 weeks and then 2 G daily for another 4. This treatment was successful.

Anaerobic Gram positive cocci These were isolated from a patient treated by Schwarz and Lazarus (1950). This patient was cured by penicillin and bacitracin.

Infection due to a Gram negative bacillus This was isolated from 4 patients by Hunter (1947). The sensitivity of the organism to streptomycin varied between 1 and 3.75 μg per ml. Treatment with 2.5 to 3.2 G of this antibiotic daily resulted in recovery in 3 of the patients.

E. coli infection Streptomycin was found to be effective in 2 of 4 patients treated by Love, Powell, Strunk, and Hinkens (1949). The organism in one patient was only inhibited by 4 μg per ml, but was none the less controlled by treatment with 3 G of streptomycin daily, together with penicillin and sulphadiazine. In other cases, Blake, Friou, and Wagner (1950) had found that the results from treatment with streptomycin were good.

Pseudomonas pyocyanea infection Treatment has rarely been successful in endocarditis due to this organism. Polymyxin E does not appear to have been tried as a method of treatment, but neomycin was given to 1 patient in whom the infecting organism was inhibited by 0.5 μg per ml of the antibiotic. The drug was given in doses of 0.5 G intramuscularly every 6 hours for 14 days. Although the patient lost his hearing for higher frequencies, he survived (Kenoyer, Stone, and Levin, 1952).

Haemophilus infection Several antibiotics have been used successfully in this condition. Wilson (1948) and McCoy and Meyer (1948) had success with streptomycin therapy alone, and Geraci (1952) found that oxytetracycline together with dihydrostreptomycin were effective when the organism was inhibited by 1.5 μg per ml of oxytetracycline. In patients described by Goudie and Lowther (1951), Hunter (1947), and Ohnger (1948) the blood stream was cleared of *Haemophilus para influenzae* by streptomycin alone.

Salm typhimurium infection De Swiet (1949) succeeded in sterilizing the blood and the vegetations of a patient infected with this organism by giving 4 G of chloramphenicol 3 hourly followed by 1.5 G 3 hourly. Death occurred none the less but was due to a number of infarcts in a flabby heart muscle. Blood cultures of another patient were cleared of infection but only after dihydrostreptomycin and chloramphenicol were administered. Treatment, however, was stopped too soon, the infection recurred, and the patient died (Stumpe and Baroody, 1951).

Brucella infection A single case, treated from the start with streptomycin and 6 G of sulphadiazine daily, recovered (Spink, Hall, Shaffer, and Braude, 1948). Hart, Morgan, and Lacey (1951) attempted to cure a patient with a *Br. abortus* infection by giving increasing doses of chlortetracycline and chloramphenicol, to both of which the organism was sensitive. This combination was followed by streptomycin and penicillin, but the infection could not be controlled. In the terminal stages, chloramphenicol alone seemed to have some effect, but by this time embolic phenomena were too frequent to be controlled.

Neisseria subflava This organism was isolated from a case by McCoy and

Meyer (1948) Its sensitivity to streptomycin was 0.5 μg per ml and treatment with 2 G of this drug daily in 3 hourly doses, together with 600 million Units daily of penicillin, ended in recovery

Chromobacterium prodigiosum A case infected with this organism failed to respond to treatment (Hawe and Hughes, 1954) This is, however, a very rare form of endocarditis

Endocarditis in which the responsible organism was not isolated These cases, although they present a problem to the attending physician, seem to have had a good prognosis One patient treated with streptomycin by Wilson (1948) recovered, and 2 treated by Friedberg (1952) with chlortetracycline in doses of 3 to 4 G daily for 7 to 8 weeks also recovered Oxytetracycline was responsible for the recovery of 1 patient who also received cortisone (Donzelote, Kaufmann, and Castel, 1952) A 4th patient recovered after receiving tetracycline (Ravina, Pestel Trocme, and Albouy, 1955) In a number of cases treated with streptomycin and penicillin, the fever was controlled (Aarseth and Gaustad, 1951 Kleinfelder, 1951, Pearsall, Pillow, and Wood, 1948, and Willcox, 1950)

Conclusion

From a survey of the different antibiotics and combinations of antibiotics which have been responsible for recovery in the cases mentioned above, apart from penicillin there seems little to choose between the various drugs Some reliance should be placed on sensitivity tests but, in a condition with such serious consequences, the most delicate test is required For this a serial tube dilution test is the most appropriate, particularly when a combination of antibiotics is to be tested (see Jawetz *et al*, 1955) A second consideration to be borne in mind is the size of the dose Bactericidal concentrations are almost always higher than those which merely inhibit bacterial growth, so that the highest dose compatible with the patient's tolerance should be chosen With penicillin, where the upper limit of toxicity in man is not yet known, 20 000 Units 2 to 3 hourly was at one time sufficient to cure some patients, but since that time doses amounting to 100 million Units daily have been employed when considered necessary With the tetracyclines, doses of 3 to 4 G a day by mouth, given at 6 hourly intervals are seldom well tolerated and, in consequence intravenous administration should be considered Streptomycin, although seldom free of vestibular consequences when injections amounting to more than 1 G daily are given, can be administered in doses of 2 G per 24 hours with only temporary impairment to the 8th nerve so long as treatment is limited to 3 weeks Erythromycin is again a drug which easily gives rise to gastrointestinal troubles when administered orally Intravenous administration is therefore to be considered in patients who cannot tolerate the drug by mouth

As regards the newer antibiotics, they have not been tested long enough to allow one to assess their dependability in treating endocarditis Novobiocin is a possible therapeutic agent in the treatment of this disease Vancomycin and ristocetin have had remarkable and encouraging effects in a few cases When all suitable antibiotic therapy has been given, however, it is well to remember that no more can be expected of antibiotics than the control of the infection The sequelae of endocarditis inherent in the process of healing

still remain a problem ruptured or scarred and calcified valves causing congestive heart failure, sclerotic kidneys following renal emboli, and cerebral embolism are still responsible for a great deal of morbidity and for death among patients. The late results after treatment are illustrated in a series of 19 cases described by Henriksen and Neukirch (1955). Thirteen of these 19 patients were discharged from hospital as bacteriologically cured, but of the 10 followed up for $2\frac{1}{2}$ to 7 years, 2 had cardiac insufficiency, 1 spastic hemiparesis, 1 mitral stenosis which was still compensated, and 4 led normal lives. Kaipainen and Seppala (1957) supported the view that the immediate survival rate gave a much more optimistic outlook than that assessed 4 years after the onset and treatment of the disease.

Infections of the Respiratory System

Upper respiratory tract infections

It is these common ailments which give rise to most errors in antibiotic treatment. The common cold, with a sore throat, even when it is accompanied by pyrexia, is more often due to an insusceptible virus than to a bacterium. Only when the cold persists or invades the paranasal sinuses or middle ear should a secondary bacterial infection be suspected, and in these circumstances swabs of nose, throat, or ear should indicate whether or not an antibiotic is required. A recent report of the treatment of acute severe sore throat or acute otitis media in general practice seems to be at variance with this opinion. Chapple, Franklin, Paulett, Tuckman, Woodall, Tomlinson, and McDonald (1956) claimed that the number of days of illness in patients given oral penicillin was reduced to nearly one half of those in patients receiving a placebo only. That results of treatment were similar irrespective of the isolation of haemolytic streptococci from the throats is an indication not of their absence, but of the physicians' care in clinical diagnosis, for the infection arises within the tissue of the tonsil rather than on the surface where streptococci are only present when they are extruded with the exudate from the crypts. When the clinical appearance of the throat is indicative of a streptococcal infection, penicillin should be administered whether the cultures from the throat swab are positive for *Str. pyogenes* or not. Treatment should be continued, either by mouth or intramuscularly, for at least 6 days to ensure that there are no recurrences. An earlier investigation carried out by Jones, Bigham and Manning (1953) on 150 patients with respiratory tract disease tested the relative effects of 600 mg. of aspirin, 500 mg. of oxytetracycline, and 200 mg. of erythromycin repeated 6 hourly by mouth. Patients with positive serological tests for viral infection were omitted from this series. The average number of hours which patients took to become afebrile with the three drugs were 30, 41, and 42 respectively. If it is legitimate to draw conclusions from these two separate groups of investigators, it would seem that penicillin still remains the antibiotic of choice in severe infections of the upper respiratory tract.

Occasionally the reaction to the streptococcal infection is so intense that penicillin alone cannot control it. In such a case cortisone may be of benefit. In a patient described by Doerner, Naegle, and Regan (1951) the infection became generalized and serous effusions occurred in joints and pleural and pericardial spaces. A raised eosinophil count was present. This patient

showed little response to penicillin and chlortetracycline or to streptomycin, but became afebrile within 12 hours once 100 mg of cortisone had been given. This dose was continued daily for 4 days, and was then reduced to 25 mg a day for 28 days. By this time the patient had fully recovered from the allergic reaction as well as the haemolytic streptococcal infection.

When choosing an antibiotic for streptococcal infections it is well to remember that haemolytic streptococci are not uniformly sensitive to penicillin. *Str. pyogenes* or β haemolytic streptococcus belonging to Lancefield group A, the common cause of 'streptococcal throat', is an organism whose susceptibility has varied little in the years during which penicillin has been used in the clinic. When a haemolytic streptococcus isolated from the throat does not belong to this particular group, however, it is advisable to try tests for sensitivity to various antibiotics in order to find the one which is most suitable. According to Jones and Finland (1957 c) there are good hopes that erythromycin will be an excellent substitute.

Bronchitis

A single organism is seldom isolated from the sputum or pharynx of patients suffering from an attack of bronchitis. In describing the bacteria cultivated from 225 pharyngeal swabs made from 163 patients, Prigal (1950) found non haemolytic streptococci most frequently. Others, in order of frequency, were *Neisseria catarrhalis*, pneumococci, haemolytic staphylococci, β haemolytic streptococci, *Proteus vulgaris*, diphtheroids, coliform bacilli, *Micrococcus tetragenus*, α haemolytic streptococci, *Bact. friedlanderii*, streptobacilli, and micrococci. Whether any of these bacteria or none of them is responsible for the patient's condition is in doubt, but it is certain that any antibiotic, which is inevitably selective, will encourage the growth of those organisms which are not susceptible to it. Bloomfield (1951) pointed out how an infection due to *H. influenzae* could by the 4th day be superimposed on a broncho-pneumonia treated with penicillin. Frequently the coliform flora multiply under antibiotic therapy although they are seldom isolated in routine cultures of normal respiratory passages. Although these changes in the throat flora may be harmless in some circumstances, they may produce serious consequences when they occur on already damaged mucous membrane. For example, a patient to whom penicillin and streptomycin were administered for several days, after pneumonectomy, developed a semi-purulent bronchitis on the 3rd day. The sputum culture produced a pure growth of coliforms. Although these organisms were highly sensitive to oxytetracycline and chloramphenicol they were not removed from the sputum in spite of large doses of these antibiotics over 5 weeks. Eventually, when the bronchitis cleared, the coliforms also disappeared. Prigal (1950), who considered that the treatment of respiratory tract infections was best carried out by means of aerosols, found that in all the cultures made in his series the majority of organisms were inhibited by a combination of penicillin and bacitracin, or of these 2 drugs together with streptomycin. So long as an aerosol method of administration is adhered to, this combination should be a good one. Nevertheless, patients were apt to have recurrent attacks in spite of the good immediate response to treatment. This Prigal ascribed to reinfection within a family, for he frequently found similar organisms in the throats of members of one family, the

most common ones being haemolytic staphylococci, β haemolytic streptococci, and pneumococci

There is one organism which Prigal (1950) did not find which has now become particularly prevalent This is *Haemophilus influenzae* Its importance has been stressed by May (1953), for of the bacteria which he was able to isolate from chronic bronchitis *H. influenzae* and the pneumococcus were the only ones consistently associated with the presence of pus in the sputum In some cases *Staph. aureus* and Friedlander's bacillus were also associated with a purulent sputum By eliminating the organisms from the sputum one by one by means of chemotherapy, May concluded that there was no correlation between the presence or absence of pus and the presence or absence of *Str. viridans*, *Neisseria catarrhalis*, *Staph. albus*, or non haemolytic streptococci For *Haemophilus influenzae* infections chloramphenicol is a most active agent when given in divided doses amounting to 2 G daily for up to 14 days (Mulder, Goslings, van der Plas, and Lopes Cardozo, 1952) Where suitable supervision of the reaction of the patient's haemopoietic system to the drug is not available, as when he is being nursed at home, the tetracyclines are useful for this infection (Romansky and Kelser, 1952), and erythromycin has also proved its effectiveness (Romansky, Nasou, Davis, and Ritts, 1956) In view of the gastro intestinal disturbances produced by these drugs, however, doses of 0.25 G 6 hourly should be tried, together with 380 mg of sodium metaphosphate, in preference to 0.5 G 6 hourly, which was the dose most commonly used in early clinical trials A combination of antibiotics is to be considered in less responsive conditions where a number of organisms are considered responsible Lepper, Kofman, Blatt, Dowling, and Jackson (1954) used the tetracyclines with or without penicillin and erythromycin in the treatment of bronchitis in patients suffering from polyomyelitis who had had tracheotomy performed on them Yet this is not without its risks Livingstone, Austen, and Kunz (1957) used combinations of penicillin, streptomycin, and tetracycline for the treatment of patients with polyomyelitis undergoing tracheotomy Serial cultures in cases other than tracheotomy revealed that 9 different species of bacteria appeared during the period of prophylaxis, much the most frequent organism being *Staph. aureus*, resistant to many antibiotics Accompanying these flora in a majority of patients was a pulmonary infection

Chronic infections of the bronchi, chronic bronchitis, and bronchiectasis

When bronchitis becomes chronic and the mucosa is already damaged, many bacteria invade the bronchi and it is seldom possible to tell which are mainly responsible for the condition Helm, May, and Livingstone (1954) considered that *H. influenzae* was chiefly responsible at the time of their report May and Oswald (1957), however, pointed out that single specimens of sputum may be misleading for in one lump of pus one type of bacterium may grow freely, while in another this organism may be entirely absent Assessment of progress under treatment therefore depends more on clinical than on bacteriological evidence Nevertheless, by repeated examination May and Oswald (1956) were able to isolate *H. influenzae* from the sputum of every patient in their trial of oxytetracycline and tetracycline over the winter months of 1955-6 On a regular dose of 0.5 G three times a day, or as near to this amount as patients could stand, 22 out of 37 patients with chronic

added infection with penicillinase-producing staphylococci. However, relapses occurred and had to be treated as on the first occasion. From this method of management Finke claimed that he markedly reduced the loss of working time. In a representative series of 51 cases, whereas 9 had previously been disabled, 2 only remained so, and while 26 patients before treatment had lost 8 days or more per attack, only 11 did so after treatment, and the number of those who did not have to absent themselves from work for 8 days or more had risen from 16 to 38. Complications which followed treatment included pneumonia in 3 patients and suppurative otitis media in another—a child. Reactions to penicillin occurred in only 13 patients, in spite of the fact that asthmatics were included in the series. In 3 per cent the reaction involved immediate shock following an injection, while the remainder suffered from urticaria, angioneurotic oedema, or other less serious symptoms. There were no blood dyscrasias, but gastro intestinal symptoms due to the tetracyclines were troublesome. Finke (1954) contended that the average patient with a bacterial respiratory infection faces a smaller risk to his health from antibacterial medication than from the liberal use of certain symptomatic remedies.

Studies of the comparative effectiveness of antibiotics in dealing with chronic infections of the bronchi were undertaken by Douglas, Somner, Marks, and Grant (1957) and by a subcommittee of the Medical Research Council (1957 a). In each trial the antibiotics chosen for trial were penicillin and oxytetracycline. Douglas *et al* also treated a series with chloramphenicol. Treatment was by intramuscular injection or by mouth. The series of patients treated by Douglas *et al* comprised both chronic bronchitis and those with bronchiectasis. Postural drainage and percussion of their chests was part of their treatment. Each patient received a short course of penicillin intramuscularly amounting to 1 or 2 million Units, and only if this failed to clear the pus from the sputum did they receive chloramphenicol or oxytetracycline, 0.5 G by mouth every 6 hours. The series, therefore, were not strictly comparable. Nevertheless, the results were not in favour of penicillin as judged by the effect on the purulence of the sputum. The percentages of patients who made a complete response to treatment or failed to do so were as follows:

| Treatment | Condition | Percentage who | | |
|--------------------------------|--------------------|----------------|----------------------|-------------------|
| | | No of patients | responded completely | failed to respond |
| Penicillin (1-2 million Units) | Bronchiectasis | 42 | 21 | 57 |
| " " " | Chronic bronchitis | 89 | 53 | 33 |
| Chloramphenicol | Bronchitis or | 23 | 78 | 22 |
| Oxytetracycline | Bronchiectasis | 28 | 39 | 43 |

Unquestionably chloramphenicol appears from these figures to be the most suitable drug. Moreover it took less time to convert the sputum to a mucoid consistency than did oxytetracycline.

Taking the trial conducted by the Medical Research Council (1957 a) as a final comparison, there was no preliminary weeding out by 1 antibiotic and all patients were suffering from bronchiectasis demonstrable by bronchograms, with symptoms for at least 2 years. Postural drainage was practised by all patients and each was allotted to his or her type of treatment in a strictly random manner. Two capsules containing 250 mg of penicillin,

oxytetracycline, or lactose were given to each patient to be taken 4 times a day on 2 days a week at home. Results were assessed at the end of a year and can be tabulated as below

| | Lactose | Penicillin | Oxytetracycline |
|---|----------|------------|-----------------|
| No of patients* | 36 | 36 | 40 |
| Final percentage of original volume of sputum | 76 | 74 | 64 |
| Percentage of patients with severe cough reduced from | 37 to 25 | 40 to 20 | 34 to 10 |
| No of patients with haemoptyses | 22 | 25 | 16 |
| Percentage with dyspnoea on exertion reduced from | 41 to 32 | 50 to 39 | 42 to 28 |
| Percentage working days lost in year | 25 | 21 | 19 |

* Patients excluded 3 who died, 1 in each group, 1 who died from a congenital abnormality, 2 who were unable to tolerate the drugs (penicillin and oxytetracycline), 4 who defaulted in lactose and oxytetracycline groups

Although the differences are not very great, yet they are all in favour of oxytetracycline. Its great disadvantage is its cost. For this reason the intermittent treatment begun at the onset of each chest cold and prescribed by Elmes *et al* (1957) has much to recommend it.

The proper treatment of chronic bronchitis, again according to Finke (1952), is to clear up completely the acute respiratory infections of childhood. Finke considered that prolonged treatment at this stage should prevent the persistence of residual infection and consequently the irreversible changes in the bronchial mucosa which make it liable to repeated infections. Whether this is so, or whether the chronic infection is the result of a congenital anomaly, there is no doubt that the early arrest of supervening infection is the best treatment of the chronic condition. In any type of treatment where prolonged use is made of one drug, one must bear in mind the possibility that infection resistant to chemotherapy may in time arise. In treating 15 patients suffering from chronic bronchitis and bronchiectasis with intravenous tetracycline for 5 to 10 days only, Fox, Dowling, Saxton, and Melody (1957) observed changes in the flora of the sputum. Pathogens such as *H. influenzae*, pneumococci, and β haemolytic streptococci disappeared in every case, but in the sputa of 2 patients they were replaced by *Staph aureus*. Although the purulence of the sputum cleared in other patients, it persisted in one where *Staph aureus* had appeared. It is possible, therefore, that prolonged treatment might bring about a state at least as severe as that from which the patient originally suffered.

On occasion the oedema and congestion in the region of the glottis may lead to an acute respiratory emergency for which drastic measures are necessary. Such conditions were described by Cardon, Lemberg and Greenebaum (1951) in 8 different cases. These authors recommended the following treatment once the condition had been differentiated from left ventricular failure or pneumonia. The tracheo bronchial tree should be aspirated repeatedly, and oxygen, a bronchodilator and vasoconstrictor, and antibiotics should be administered. Penicillin and streptomycin were used at first as an aerosol. Later they were given parenterally as procaine penicillin in a dose of 300,000 or 600,000 Units in 1 or 2 injections daily with streptomycin, the streptomycin was given in doses of 1 G a day in 1 or 2 injections. When the response

was not quick enough from penicillin and streptomycin the tetracyclines were recommended and it was claimed that, when these were given by mouth, intravenously, or even by aerosol, they improved the prognosis. In special cases bacitracin or chloramphenicol were of value. The actual results quoted, however, appear to have been obtained without these latter antibiotics. Of 4 cases treated by the various methods described above, but with the exclusion of antibiotics, immediate relief and recovery followed in 1 only, of the 4 to whom penicillin and streptomycin were administered 3 recovered, and the 1 who died had received penicillin by mouth only. Barach (1951) also used antibiotics as well as bronchodilators, cortisone, and mechanical measures to control bronchial asthma and emphysema. Barach found the tetracyclines or chloramphenicol particularly valuable in the acute respiratory infections which occurred in these patients, but he relied on penicillin for prolonged treatment. The most difficult infections to eradicate at that time were those due to proteus or monilia, nystatin was not at that time available.

For asthma with bronchitis Finke (1954) gave cortisone with the antibiotics. One hundred mg. were administered daily by mouth with gradual reduction of the dose to 25 to 50 mg. in 2 weeks, the dose was gradually reduced still further until it was discontinued. Reactions to the cortisone undoubtedly occurred, for example, oedema or hypertension in adults, and weight gain, gastric disturbances, and enuresis in children, and skin reactions and gastric upsets followed the antibiotic treatment. Nevertheless, the results were sufficiently gratifying to justify the treatment. Of 78 patients receiving therapy over 2½ to 5 years, results were considered good or excellent in no less than 80 per cent. Once it had been discontinued the cortisone was not again required for 9 months or more. Thus the working capacity of these chronic sufferers was much improved. It was pointed out that much better results were obtained in the younger, and less chronic, patients. Finke's findings were confirmed in part in a smaller series by Savidge and Brockbank (1954).

In patients with bronchiectasis the object of treatment is somewhat different from that in chronic bronchitis with asthma. If possible, the offending lung is resected but before this operation is performed eradication of the bronchial flora is aimed at, rather than relief of the acute exacerbation. Elimination of all the flora present in the bronchial secretions should entail the administration of a succession of antibiotics. Nevertheless, it is interesting to note the observations of Wynn Williams (1953) on a 5 year survey of a population of nearly 150 000 in and around Bedford, England. Amongst these people 214 were found to have bronchiectasis, yet in all these cases resection had been carried out in 6 only, where there was bilateral disease or complications from the bronchiectasis. The patients had been treated by penicillin, streptomycin, and/or chloramphenicol, and of the 6 deaths which had taken place in the previous 3 years, none had been from pneumonia or other infective complications. The condition of the other patients had remained stationary. Wynn Williams concluded that a high proportion of bronchiectatics are likely to remain well and be little inconvenienced if they persevere with postural drainage and antibiotics. In another small series of patients with chronic and advanced respiratory infections, Helm, May, and Livingstone (1954, 1956) reported results soon after beginning treatment and

2 to 3 years later In this series oxytetracycline was used Briefly the results were as follows

| Condition | No of cases | No on treatment 1954 | No on treatment with benefit (approx 2 years) 1956 | Cause of abandoning treatment |
|----------------------------------|-------------|----------------------|--|---|
| Chronic staphylococcal pneumonia | 1 | 1 | 0 | Died after 3 years |
| Bronchitis | 17 | 9 | 5 | Autogenous vaccine required—2 Recurrent diarrhoea—1 Relatively slight benefit—1 |
| Infective asthma | 13 | 0 | | |
| Bronchiectasis | 7 | 4 | 3 | Relatively slight benefit—1 |
| Totals | 38 | 14 | 8 | |

These figures do not look particularly encouraging In the one patient suffering from chronic staphylococcal pneumonia benefit was at first obtained from oxytetracycline, but eventually a resistant staphylococcal infection supervened, and deterioration occurred, despite increase in the dose of the drug A combination or succession of drugs thus seems to be indicated

It would thus appear that surgery is now indicated, not to remove irremediably infected areas but to remove structural abnormalities which interfere with respiratory function Thus chronic fibrotic changes may lead to embarrassment of the heart or to difficulty in aeration of the remaining healthy tissue Both of these conditions may be improved by removing all or part of the offending lung

Abscess of the lung

The organisms usually found in acute lung abscess are spirochaetes, fusiform bacilli, vibrios, and/or Gram positive anaerobic cocci (Shoemaker, Yow, and Byrd, 1955) All the organisms are sensitive to penicillin and this drug has been administered from 1943 onwards for this condition (for example, Drake and Sones, 1951) Undoubtedly penicillin reduced the incidence of and mortality from lung abscess (Blades, 1947, Neuhof, 1945 Waterman and Domm, 1954 Weingartner, 1955) The addition of streptomycin was also of benefit in many cases There were still cases however, which became chronic and were refractory to treatment In these the tetracyclines or chloramphenicol have often been invaluable, for example, in the 2 cases described by King Lewis, Welch, Clark, Johnson, Lyons, Scott, and Cornely (1950) Whether or not lung abscesses are amenable to any particular antibiotic now depends largely on the results of the sensitivity tests of the bacteria involved Given an effective antibiotic, healing will none the less depend not only on the administration of a suitable dose, parenterally, by mouth, by aerosol, or by instillation, but also on suitable drainage either by posture or by surgical means When the infection has been eliminated there remains the question of removing the fibrotic area where the abscess was originally situated Lobectomy or resection of part of a lung is only indicated when the structural deformity interferes with the proper functioning of the lungs Empyema,

bronchopleural fistula, post operative pain, and brain abscess are still complications which cannot always be avoided (Shoemaker *et al*, 1955) but they seldom cause death when diagnosed in time. The treatment at present advised is that of Shoemaker *et al* (1955) aqueous penicillin in doses of 100,000 to 300,000 Units 4 hourly by intramuscular injection, followed, after definite clinical improvement has occurred, by a long acting preparation of penicillin. Weekly bacteriological examinations of the sputum and sensitivity tests should be made so that another antibiotic to which the organisms are susceptible may be used when penicillin resistant organisms appear. The patient should be confined to bed until afebrile and progressing favourably. With these methods, and bronchoscopy when postural drainage did not appear adequate, the 67 patients treated by these authors between January 1950 and December 1954 were febrile for an average of not more than 8 days, and they remained in hospital for an average of only 34 days. Although complications occurred, even metastatic brain abscesses, all patients fully recovered with or without operation. The figures given by Shoemaker *et al* (1955) coincide remarkably well with those of Gittens and Mihaly (1954). These authors described 37 patients with acute lung abscess. Those treated by penicillin recovered in an average of 35 days, those treated by penicillin and sulphadiazine in 67.7 days, and those treated by one of the tetracyclines in 64 to 69 days. The results of this series of cases, though small, are once more in favour of penicillin.

Bacterial pneumonia

Since the responsible organism in bacterial pneumonia is most commonly the pneumococcus, which is one of the most sensitive of bacteria to antibiotics, there are few antibiotics to which pneumonia does not respond. Since it has no unpleasant gastro intestinal consequences and acts rapidly, penicillin is preferable to the tetracyclines or chloramphenicol, but there is little difference in the therapeutic results (Weiss, Eisenberg, Alexander, Mann, and Flippin, 1954). As with all conditions discussed in this section, the advisability of serial examination of the exudate, in this instance the sputum, is emphasized. With penicillin treatment the pneumococcus may be replaced by a penicillinase producing staphylococcus or by Gram negative bacteria, in which case a tetracycline is indicated. A resistant infection may also supervene in cases treated initially with the tetracyclines. When this occurs the appropriate antibiotic for the predominant organism is indicated. The use of antibiotics has not only lowered the death rate since the sulphonamide era (Dowling, 1956) but deaths now are not so much caused by the primary infection as by the damage caused to the heart and other organs, especially in elderly people. Deaths may be due to congestive heart failure, auricular fibrillation, cerebral haemorrhage, bronchiectasis, delirium tremens, diabetic coma, uraemia, or cirrhosis of the liver, rather than to the infection itself (Dowling and Lepper, 1951).

Staphylococcal pneumonia

More than in any other infection, sensitivity tests are needed for the responsible staphylococcus. Experience is not uniform in the results obtained from certain antibiotics. For example, of 15 cases occurring in Ulster, after an outbreak of influenza, 6 recovered on penicillin alone, 3 on streptomycin

following penicillin, and one on chlortetracycline following penicillin (Grant and Barber, 1954). Of late years increasing attention has been drawn to the prevalence of pneumonia caused by antibiotic resistant staphylococci. Hausman and Karls (1956) described 18 such cases in a consecutive series of 122 cases of pneumonia admitted to one medical unit during 1952 to 1954. Though no epidemic of influenza prevailed in the area from which the patients were drawn, the cases were noted for the severity of their infection. All of the staphylococci tested (by the disk method) were resistant to 1 to 5 Units per ml penicillin and the results of treatment indicated that in 3 other patients the staphylococcus was resistant. Fortunately all eventually recovered on treatment with a tetracycline or erythromycin. The following year Gresham and Gleeson White (1957) described the pathological findings in 14 cases of fatal staphylococcal pneumonia. Pus was found in the bronchi and bronchioles and the alveoli were surrounded by haemorrhagic oedema and filled with similar fluid. Many polymorphonuclear cells and clumps of Gram positive cocci were found in the affected bronchioles and alveolar ducts. Cultures not always showing a pure growth of staphylococci contained organisms which were resistant to penicillin, streptomycin, chlortetracycline, and, in nearly every case to chloramphenicol. One organism was resistant to erythromycin. These authors stated that in few of these patients was the infection suspected during life as they were already ill from other causes such as coronary thrombosis, bronchial carcinoma, chronic tetanus, general peritonitis, &c.

Again Beaven and Burry (1956) described an epidemic of staphylococcal pneumonia and empyema in newborn infants following minor superficial infections in which 8 out of 11 infants died. The staphylococci were all found to be resistant to penicillin and to show varying degrees of resistance to streptomycin, chlortetracycline and oxytetracycline, and to chloramphenicol, but all were sensitive to erythromycin.

The problem in these cases is in making a speedy diagnosis. With a culture of the staphylococcus in hand it should not be difficult to find the antibiotic or combination of antibiotics inimical to its spread.

Klebsiella pneumoniae

Although rare, pneumonia due to Friedlander's bacillus occasionally occurs. For this condition, streptomycin together with sulphonamides, penicillin, chloramphenicol or one of the tetracyclines is indicated (Gill, 1951, and Kirby and Coleman, 1951). *Unhappily*, even with the help of antibiotics, the prognosis is not good. Jervcy (1957) stated that the mortality was still about 50 per cent. He recommended the administration of sulphonamides, streptomycin and tetracyclines. Limson, Romansky, and Shea (1956) in describing the outcome in 13 patients with the acute disease seen over 2 years found that 9 had died, all of whom were alcoholics. Of the 4 survivors 2 received tetracycline alone or with streptomycin and later chloramphenicol, while the 1 serious case succumbed after receiving, in succession, penicillin, streptomycin and chlortetracycline. Another case, a chronic alcoholic of 79 years, who had meningitis as well, was treated successfully by Sterkel and Knight (1957). No new antibiotic was introduced into his treatment for he was given sulphadiazine, penicillin and streptomycin, but he also received cortisone. Whether because of or in spite of this last drug he fully recovered.

From these reports there is no clear cut evidence that any single antibiotic

is outstanding in its effect on *Klebsiella pneumoniae*. Some indication has been given by Weiss, Eisenberg, Spivack, Nadel, Kayser, Sathavara, and Flippin (1956) that studies *in vitro* favour streptomycin and chloramphenicol.

When faced with the immediate treatment of a patient suffering from pneumonia, the physician finds too many antibiotics available rather than too few. The study carried out for the Medical Research Council in London hospitals, from February 1950 to August 1951 in 267 cases of pneumonia, had as its object an assessment of the relative value of treatment by penicillin alone and by penicillin together with sulphonamides, chlortetracycline, or chloramphenicol, irrespective of the aetiology of the disease. The results, as far as mortality was concerned, were in favour of penicillin alone, and they were very definitely in its favour from the point of view of economy of expenditure (Medical Research Council, Report to 1951). Thus, although a physician may need to change his treatment in the light of laboratory findings, penicillin would seem to be the first choice for the treatment of bacterial pneumonia.

Atypical pneumonia

Primary atypical pneumonia has already been dealt with under viral infections. Much work has been done on comparative trials with different antibiotics. As late as 1954 conclusions were still being drawn about the greater effectiveness of chloramphenicol or the tetracyclines compared with penicillin. Tetracycline was considered the most suitable of these drugs, but the evidence did not amount to a demonstration that any of these agents were specific for the infection in question (Graves and Ball, 1951, Gallagher and Gallagher, 1952, Mecklejohn, Thalman, Walgora, Kempe, and Lennette, 1954, and Peck and Berry, 1951). Studies elsewhere than in the United States were not unanimously in favour of antibiotic treatment for this condition (Berglund, 1954, and Hilden and Norregaard, 1950) and opinions even about the antibiotics were also divided (Finland, 1953). The difference between controlled and treated cases increases with the severity of a lesion. Walker (1953) therefore selected from his series only those patients with radiological evidence of pneumonia and charted the incidence of cough and fever during and after therapy. Fig. 13 shows how closely these approximated one another whether the patients received a placebo or chlortetracycline.

Infections within the Abdomen

Pre-operative preparation

The main surgical interest in the use of antibiotics for the alimentary canal has been in reducing the bowel flora so as to render contamination of the wound or the peritoneum a less serious risk. The requirements of an antibiotic for use in bowel surgery are that it should reduce the flora of the gut without inducing resistance in the organisms, that it should exert its effect over a reasonable period of time, and that it should not irritate the mucosal lining of the gut. Since 1950, numbers of comparative trials have been carried out in the hope of finding the ideal antibiotic or combination of antibiotics for this purpose. In 1950 chlortetracycline and oxytetracycline, together with vitamin supplements daily for 4 to 5 days, were considered adequate to keep patients afebrile after operation in spite of contamination of the peritoneal cavity with faeces (Andina and Allemann, 1950). Comparison of the bacterial

flora of the stools in patients treated with chlortetracycline, chloramphenicol, the sulphonamides, or dihydrostreptomycin by mouth favoured chlortetracycline, but even with this drug a not inconsiderable number of resistant organisms were found in the stools. Among these were *Ps aeruginosa*, *B proteus*, *Str faecalis*, *A aerogenes*, coagulase negative micrococci, aerobic spore formers, and yeasts. Whether or not the bacteria developed resistance after administration of the drug, as in the case of dihydrostreptomycin, or were resistant from the beginning of treatment, none of the agents was ideal

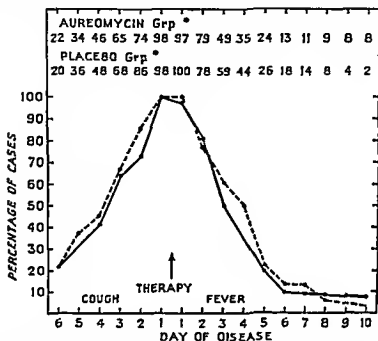


FIG. 13 The course of primary atypical pneumonia as demonstrated by incidence of cough before and fever after beginning of therapy in young adults with radiologic evidence of the disease

* No. of cases (diagnosis by exclusion of other diseases or by 4 fold rise in cold haemagglutination titre from acute to convalescent stages. Treatment—Aureomycin 0.5 G 6 hourly till the temperature was normal for 2 days or by yellow placebo capsules in the same dosage

(From Walker Amer J Med 1953 15, 593)

(Dearing and Heilman, 1950) When bacterial counts rather than the presence or absence of certain organisms were the criteria used, it was found that the administration of penicillin parenterally in doses of 500,000 to 1 million Units daily produced a consistent rise in the number of aerobes present, particularly *E coli*, but had no appreciable effect on the frequency or character of the stools as was the case with chlortetracycline. On the other hand, 2 G of streptomycin daily greatly reduced the number of aerobes present over the first 3 days of administration and the tetracycline (1 to 2 G daily) reduced the number of both aerobes and anaerobes (Marshall, Palmer, and Kirsner, 1950). Pulaski, Connell, and Seeley (1950) first drew attention to the possible clinical significance of bacteroides, which could be isolated from the stools only with some difficulty. These organisms are apparently responsible for the

foul odour of the stools. Of 4 varieties tested, 3 were particularly sensitive to penicillin and a fourth to oxytetracycline (Garrod, 1955). However, in general, more of these organisms in different sites could be controlled by one of the tetracyclines (Finegold and Hewitt, 1956, Lodenkamper and Stienen, 1955). According to Garrod (1955), those varieties which are sensitive to penicillin were most commonly found in the upper part of the alimentary tract, those most sensitive to tetracycline in the lower bowel. It is possible that these bacteria, which are sometimes responsible for various septic conditions ranging from local necrosis complicating gingivitis to septicaemia, may need to be controlled more often than might be suggested by their relatively infrequent isolation.

The question now to be discussed is whether a combination of antibiotics is more efficacious in reducing the potentially pathogenic bowel flora than 1 antibiotic alone. Experimental work on dogs in which peritonitis was induced by injecting some bowel contents into the peritoneal cavity, after excision of a section of the colon, and subsequent anastomosis of the bowel showed that pre-operative treatment with each of these antibiotics singly resulted in the survival of all of the animals only in the case of oxytetracycline, but penicillin, when given together with polymyxin or chloramphenicol, was also capable of preventing death (Edmiston, Davies, Ledwich, Marchi, Sbafer, and McCorkle, 1951). In man, a combination of streptomycin, in doses of 330 mg 4 hourly given by mouth with aluminum pectinate, and bacitracin, in doses of 50 000 to 75 000 Units also given by mouth 4 hourly, reduced the number of coliforms, *Str. faecalis*, and spore formers (Finegold, 1951). Neomycin and polymyxin B reduced the number of coliforms, enterococci, and anaerobes but did not affect yeasts, staphylococci, or chromobacteria (Jawetz and Bierman, 1952). Neomycin together with bacitracin reduced the numbers of all the organisms in the intestine except some yeasts and bacteroides (Fog 1954). During 1953-4 oral administration of neomycin came into favour in the clinic and was used in combination with various antibiotics such as erythromycin, carbomycin, and oxytetracycline. With all these combinations the number of Gram negative and Gram positive bacteria, aerobes and anaerobes were reduced, but yeasts still persisted, and enterococci flourished when neomycin was used with oxytetracycline (Prigot, Shidlovsky, Turell, and Marmell 1955 a and b). In spite of these unfavourable bacteriological findings, an enthusiastic interpretation of the results of another combination of neomycin with a tetracycline was made by Milberg, Kamens, Rapstein, and Banowitch (1954-5). It was found that rectal swabs could not be sterilized with chlortetracycline or neomycin alone, but in 30 out of 50 patients treated with a combination of these 2 antibiotics administered in doses of 0.5 G and 0.25 G respectively every 6 hours for 2 days, sterilization was achieved. There were no post-operative complications in these patients and the average number of days during which fever persisted amounted to 1.46 compared with 2.37 and 7.95 when each of the antibiotics was used alone. These satisfactory results were confirmed a year later (Milberg, Kamens, Leucowicz, and Jampol, 1955-6) when the effects of different doses were compared. Neomycin (0.5 G) together with oxytetracycline (0.25 G) 6 hourly by mouth for 2 days sterilized the faeces in two thirds of 90 patients treated in this way. A faecal fistula and 2 wound infections complicated the convalescence in 3 patients, but even so these complications were not sufficiently

serious to raise the average number of post operative febrile days above 1.65. Further confirmation of the good results from this combination of drugs was given by Cohn and Longacre (1956) and Cohn (1957), who considered neomycin together with tetracycline to be the most satisfactory combination of drugs for preparing the colon for surgery that they had tried. Coliforms, clostridia, and bacteroides all disappeared as a result of this treatment and streptococci, enterococci, and staphylococci were not found in the colon in the majority of patients. Poth (1957) questioned the advisability of employing a tetracycline on the grounds that its ready absorption did not confine its action to the gastro intestinal canal and of its liability to irritate the intestinal mucosa. He, like Fog (1954), found a combination of neomycin with bacitracin most suitable, or, failing bacitracin, *Soluthiazole* emphasizing that their administration should cease during the stage of post operative paralytic ileus. On opening the peritoneal cavity, therefore, he placed 200 ml. of a 0.5 per cent. solution neomycin containing 500 Units of bacitracin per ml., or injected up to a litre of this solution through the abdominal wall half an hour before operation. He claimed that, after this time, no viable bacteria could be cultivated from the peritoneal contents. Poth's recommendations may be admirable, but in following them one must take care not to overstep the limit of dosage of neomycin which he sets. Cases of apnoea have followed intraperitoneal instillation of neomycin, some of which have not survived. Needless to say the proper action of antibiotics is only achieved by maintaining a gastro intestinal canal as free as possible of detritus. This is usually best obtained by preceding their administration by saline laxatives or castor oil and providing, during it, a low residue diet.

Diarrhoea and vomiting in infants and children

There is no doubt that many of these conditions are caused by salmonellae or shigellae but since in some localities facilities are not available for distinguishing one aetiological organism from another, or treatment may appear necessary before the bacteriological diagnosis is made, it is of interest to observe which antibiotic produces the best results. A heterogeneous collection of patients was described by Aguilar and Olarte (1950). Three hundred and sixty one children were treated in Mexico, the predominant organisms isolated eventually being shigellae, salmonellae, proteus, or coliforms. The children were treated with sulphadiazine, streptomycin, chlortetracycline, or chloramphenicol. Since sensitivity tests were carried out, it is of interest to note that each of these bacteria was susceptible to chloramphenicol more often than to any of the other agents used, and that the best clinical results were obtained with this antibiotic. In the less hot climate of northern Italy, Colombo (1951) studied the mortality in children suffering from gastro enteritis treated with the sulphonamides, penicillin, streptomycin, and chloramphenicol by mouth. The lowest mortality prior to the introduction of chloramphenicol had followed the administration of penicillin, but of the 4 cases treated with chloramphenicol none had died. In a temperate climate—England—a similar investigation was carried out under the supervision of the Antibiotics Trials Committee of the Medical Research Council (1953). In this trial all salmonella and shigella infections were excluded, which considerably influenced the figures for chloramphenicol. In these patients, treated in 10 different hospitals, there was no great difference between the

results obtained in any of the groups treated with the different antibiotics with respect to the number of deaths, the average duration of the diarrhoea, or the time taken for clinical recovery to occur. However, the group treated with sulphadiazine did better than any other. In Paris, Marie, Salet, Le Minor, Berkman, and Payet (1955) had preferred to use chloramphenicol for the gastro enteritis of infancy until 6 months before making their report. At this time they changed over to neomycin for all children with stools containing *E. coli*, strain 0111. Their results were much improved and had the support of the bacteriological findings of Barr (1956), who isolated from active cases of infantile diarrhoea *Ps. aeruginosa*, *Proteus vulgaris*, and 2 strains of *E. coli*—all of which were more readily inhibited by neomycin than by any of the tetracyclines or chloramphenicol. (They were very much more susceptible to a combination of neomycin and polymyxin but there were no trials to indicate how satisfactory this mixture would be in the clinic.) Since neomycin is poorly absorbed, the dose of 50 mg per kg of body weight which was used in this trial produced no evidence of toxicity in the babies. As the aetiology of diarrhoea and vomiting in infants and children seems to vary with the locality, it would seem necessary that the choice of an antibiotic should vary also. Nevertheless, since neomycin is a bactericidal drug it would seem legitimate to begin treatment with neomycin by mouth. This may be given in divided doses amounting to 50 mg per kg per 24 hours, until the result of bacteriological examination of the stools has been obtained. This drug may be continued if indicated, or another then substituted. It must not be forgotten that framycetin in French and Belgian hands has already proved of value in the treatment of gastro enteritis due to pathogenic strains of *E. coli*.

Acute enterocolitis

Patients dying of this complaint have been found to have a massive amount of fluid in their gastro intestinal canals, often purulent and containing patches of sloughed mucosa. Many Gram positive cocci are seen in the exudate but cultures, though containing *Staph. aureus*, seldom reveal a pure growth of this organism. Although this severe complication may arise irrespective of the type of pre operative antibiotic therapy, the staphylococci isolated were found to be resistant to that being used as a pre operative measure in each of the 16 cases reported by Lepley and Smith (1957). This condition is often attributed to the use of the tetracyclines but in such cases should be differentiated from acute staphylococcal food poisoning. It may arise as a complication after operation on the alimentary canal. Some cases have been known to follow parenteral administration of penicillin and streptomycin and even of neomycin, but it is questionable whether the enteritis is then not as much the result of the operation as of the antibiotic therapy. In any event, the onset is so acute that the patient rapidly becomes shocked and dehydrated and remedial measures are required immediately and discontinuance of administration of the antibiotic in use. At the time of reporting, erythromycin has been found to be useful in this condition, for example in the cases reported from Copenhagen by Thaysen and Eriksen (1955-6), but there are also reports of cases in which erythromycin was of no avail. Novobiocin as well as erythromycin was used by Turnbull (1957), either antibiotic being injected at first intravenously for rapid effect. Because they must be given intravenously, vancomycin and ristocetin appear to have an ideal field here.

for their action. Vancomycin gave promising results in a few trials (Geraci *et al*, 1957), as also did staphylomycin (De Somer and Van de Voorde, 1957). However, it is as well to bear in mind that the newer antibiotics such as spiramycin, novobiocin, or thiostrepton may soon enter the field as reliable anti staphylococcal agents. Needless to say, when staphylococcal enteritis arises during the administration of an antibiotic this should be withdrawn immediately.

Peritonitis

Experimental and clinical results have shown that one of the tetracyclines or penicillin, together with streptomycin, is usually effective in the treatment of acute diffuse peritonitis. If peritonitis is suspected, the tetracyclines can be given by mouth, but when the patient is shocked intravenous administration is advisable (Pulaski, Noyes, Evans, and Brame, 1954, Seahury, 1955). When the aetiological organism is known, a change can be made to that antibiotic most suitable for therapy, bearing in mind that anything which tends to irritate the gastro intestinal mucosa should be avoided if possible.

Infections of the gall-bladder and biliary passages

Two hundred and forty seven cultures were made by Twiss, Carter, and Fishman (1951) from the duodenal hile, gall bladder, common duct, or cystic duct in patients with chronic cholecystitis who came to operation. The bacteria found were of the same type as those found in similar situations when there was no evidence of infection. In order of prevalence they were *E. coli*, sterile cultures, non haemolytic streptococci, *Salm typhi*, α haemolytic streptococci, *Cl. perfringens*, *Proteus vulgaris*, *Staph aureus*, *Staph albus*, *Alkaligenes faecalis*, and unidentified Gram negative rods. There was a considerable preponderance of the first 3 types. Such flora appear ideal for the administration of the tetracyclines. Moreover, tests of the excretion of tetracycline into the gall bladder hile by Zaslow and his colleagues (Zaslow, 1953, Zaslow, Cohn, and Ball, 1955, Cohn, Zaslow, Dickens, and Tumen, 1956, Cohn and Zaslow, 1955-6) showed that, when the drugs were given by mouth in doses of 0.25 to 0.5 G 6 hourly, high concentrations were found in the bile (about 25 μ g. per ml) irrespective of whether the gall bladder was diseased or not. If, however, the cystic duct was obstructed or there was derangement of the liver function, concentrations of the antibiotic were low or undetectable. Intravenous administration of oxytetracycline produced high levels in the bile 2 hours after administration, but no antibiotic was left in the gall bladder after 12 hours. There seems to be little advantage, therefore, in intravenous over oral administration except in cases with intractable vomiting.

In gall bladder disease, therefore, at present one of the tetracyclines is indicated, the dose being 0.25 G 6 hourly by mouth if the patient can take it this way. Even when obstruction exists and operation is contemplated, the administration of tetracycline is still indicated, for if the obstruction can be removed at operation the drug already in the blood stream can then be readily excreted into the bile.

Hepatitis

Experimental work has been in favour of the tetracyclines in liver disease. Rats were fed a diet low in protein and high in fat so as to produce liver necrosis, and various antibiotics as well as sulphadiazine were administered (Gyorgy, Stokes, Goldblatt, and Popper, 1951). The gain in weight of the rats over 4 weeks and their survival time were measured. It was found that the effect of chlortetracycline was equal to or greater than that of other antibiotics in inducing a gain in weight, and the animals survived for a longer period when given this drug. Oxytetracycline, although not as active as chlortetracycline, kept the animals alive for longer than the other antibiotics—streptomycin, penicillin, chloramphenicol, or polymyxin. On death, massive necrosis of the liver was observed in all the animals, but to a lesser degree in those treated with the 2 tetracyclines. When these findings were applied clinically (Ducci and Katz, 1952) acute and fulminant hepatitis was found to respond dramatically to the administration of chlortetracycline or oxytetracycline, given together with cortisone, in 2 of 3 patients. The 3rd, who died within an hour of admission, was too near death before treatment was started. When, on the other hand the disease was chronic or subacute, little or no effect was produced by the tetracyclines. Three cases of this sort were treated by Ducci and Katz (1952) without benefit. Nor did Faloon, Downs, Duggan and Prior (1957) find any significant changes in liver function after tetracycline in 3 patients with evidence of liver disease. It is possible that bacteria which are not susceptible to the tetracyclines appear readily and multiply in the gut, so counteracting the original effect of the antibiotic. In treating patients with chlortetracycline Ruebner (1957) found that whether or not patients had cirrhosis of the liver antibiotic resistant strains of faecal flora appeared in the faeces within 3 to 8 days of beginning treatment. The only germs consistently repressed were bacteroides. On the other hand, Fisher and Faloon (1957) and Dawson *et al* (1957) found persistent administration of neomycin could produce pronounced clinical benefit in patients with chronic disease. So long as the antibiotic was being taken, i.e. over periods of up to 70 months, blood ammonia levels were reduced to normal in the majority of patients. This antibiotic is therefore recommended in doses of 4 to 12 G daily by mouth.

Liver abscess

Penicillin and streptomycin were instrumental in the recovery of a patient suffering from liver abscess due to an anaerobic *Str. viridans*, the condition had originated from a ruptured gangrenous appendix. Rupture of the appendix had been followed by purulent peritonitis which became localized as abscesses in various parts of the abdomen and pelvis and eventually, in the liver. As each abscess was located, drainage was instituted, and although the illness, with intermissions, lasted over 6 months, the patient eventually recovered (Fuss and Fuhrman, 1950). Other cases of solitary pyogenic abscesses of the liver recovered when the abscesses were aspirated repeatedly and the antibiotics were instilled into the cavities (McFadzean, Chang, and Wong, 1953).

Infections of the Central Nervous System

Meningitis

In treating meningitis it is obviously preferable to minimize the number of intrathecal injections so as to limit trauma, and for this purpose chloramphenicol is particularly useful for it penetrates the blood brain barrier after oral or intravenous administration in concentrations which are comparable with those in the serum. It has also been found relatively recently that penicillin, when administered in doses of 1 million Units intramuscularly every 2 hours to patients with inflamed meninges, will penetrate the meninges in sufficient amounts to control an organism as susceptible as the pneumococcus (Dowling, Sweet, Robinson, Zellers, and Hirsh, 1949 d). In the case of streptomycin, chlor and oxytetracycline, erythromycin and novobiocin, therapeutic concentrations cannot be found so reliably in the cerebrospinal fluid after oral or intramuscular administration. It is advisable therefore to begin with a high intravenous dose. Before the causal organism is known an intravenous injection of tetracycline which penetrates the intrathecal space more readily than its analogues—75 mg per kg body weight per day—gives promise of controlling the pathogens most commonly found in purulent meningitis (Koch and Hansen, 1957). Some workers still prefer to use penicillin, sulphadiazine, streptomycin, and chloramphenicol. With combinations of some or all of these antibiotics a series of 150 children were treated at the Medical College of Virginia between January 1953 and 1956. There was a mortality of 9.7 per cent but less serious consequences, such as hydrocephalus, deafness, and paralysis of the 6th cranial nerve, were seen in 18 children (French, 1957). A still lower mortality—8 per cent—was achieved with similar chemotherapy by Smith and Herring (1953). Once a bacterium has been isolated from the cerebrospinal fluid, one has the choice of continuing with tetracycline—by mouth after 72 hours—if the patient is doing well or changing to another antibiotic. The following list gives the types of alternative treatment which should be suitable for the different kinds of infection until the susceptibility of the causative organism is known. From this moment treatment in patients who are not progressing favourably is governed by the result of the laboratory tests. In meningitis, where irremediable damage may be caused in so short a space of time, early diagnosis is important and therapy should be given as quickly as possible after diagnosis is made. When the condition is fulminating, cortisone in doses of 50 to 100 mg daily may be given or adrenal cortical extract in doses of 20 to 40 ml intravenously at once, followed by 10 ml hourly for 6 hours and then at increasing intervals (Banks, 1953, and Bartolozzi and Borcheresi, 1954). Treatment should be limited to the shortest time possible commensurate with signs of improvement. The addition of sulphonamide to penicillin in pneumococcal meningitis has been frequently used in the past but there is little evidence that so sensitive an organism as the pneumococcus requires adjuvant treatment in addition to penicillin. Because pneumococcal infection tends to induce the formation of fibrin, in which the pneumococci become enmeshed and hence to form obstructions to the free flow of cerebrospinal fluid, the complications of this form of meningitis are particularly severe. Involvement of the cranial nerves, particularly those with long courses along the base of the brain, is another common phenomenon resulting

from the formation of fibrin, and this may lead to paresis, paralysis, blindness, or loss of hearing. No antibiotic will overcome the effects of the fibrin deposit, which demands either a suitable lysing agent or treatment by ventricular or subdural taps when block prevents circulation of the fluid. Sometimes local instillations of the antibiotic are indicated when enclosed effusions are found to be infected (Kneeland, 1951, Smith, H. V., 1951, and Smith, M. H. D., Dormont, and Prather, 1951).

| <i>Infecting organism</i> | <i>Antibiotic first choice</i> | <i>Dosage</i> | <i>Route</i> |
|---------------------------|--------------------------------|-----------------------------------|--------------|
| Meningococcus | Penicillin* | 1,000 000 Units 2 hourly | I M |
| | or erythromycin | 500 mg 4-8 hourly | I V |
| <i>H. influenzae</i> | Chloramphenicol | 100-200 mg per kg of weight daily | I M |
| | or streptomycin | 200 mg per kg of weight daily | I M |
| | with sulphadiazine | | P O |
| <i>Pneumococcus</i> | Aqueous crystalline penicillin | 1,000 000 Units 2 hourly | I M |
| <i>Str. pyogenes</i> | Penicillin | 1 000 000 Units 2 hourly | I M |
| | or chlor | 100 mg per kg of weight daily | I M |
| | amphenicol | or 200 mg per kg daily | P O |
| <i>Staph. aureus</i> | Aqueous crystalline penicillin | As for pneumococcus | |
| | or erythromycin | 500 mg 4-8 hourly | I V |
| | or chloramphenicol | 100 mg per kg of weight daily | P O |
| | Streptomycin | 40 mg per kg of weight daily | I M |
| <i>E. coli</i> | | + 5 mg daily | I Th |
| | with sulphadiazine | to produce 10-15% in the blood | P O |
| | or chloramphenicol | As for <i>Str. pyogenes</i> | |
| <i>Pseudomonas</i> | Polymyxin | 2.5 mg per kg of weight daily | I M |
| | | + 2.5 mg daily | I Th |
| | or streptomycin | 40 mg per kg of weight daily | I M |
| | | + 5 mg daily | I Th |
| | with sulphadiazine | As for <i>E. coli</i> | |

* Sulphadiazine is sufficient for the great majority of cases of meningococcal meningitis.

I V = intravenously I M = intramuscularly I Th = intrathecally P O = by mouth

(After the recommendations of Alexander 1953, Hoyne, 1954, McCrumb, Hall, Imburg, Merideth, Helmhold, DeFillio, and Woodward 1951, Pease and Alexander, 1953, and Romansky *et al.* 1957.)

With the meningococcus the situation is rather different, for the organism varies enough in its susceptibility to be on one occasion more sensitive to a sulphonamide and on another to an antibiotic. There is, therefore, good reason to use both drugs together before the bacteriological diagnosis and susceptibility to penicillin are known. It is still a question whether intrathecal injection should be practised with penicillin and streptomycin as much as it has been in the past. Unquestionably there have been serious and disastrous accidents as a consequence of this procedure and it would seem wiser to reserve its use for neurological units specially trained in the technique. Various complications, such as haemorrhage in the subdural space, or collections of fluid here or elsewhere, also require a specialist's technique. When such collections appear infected and are walled off from the circulation, local instillations into them are recommended.

In meningitis due to *Haemophilus influenzae* chlortetracycline has been successful as well as chloramphenicol (Drake, Bradley, Imburg, McCrumb,

and Woodward, 1950, Lepper, Wehrle, and Blatt, 1952) The dose given was 50 mg per kg body weight per 24 hours and was administered intravenously.

Erythromycin, chlortetracycline, and oxytetracycline have been found useful in meningococcal and pneumococcal meningitis and in meningitis due to *H influenzae*. *E coli* has also responded to the tetracyclines. These drugs are to be regarded as second choices, however, because of the uncertainty of their penetration into the intrathecal space.

There is also some hope of recovery in the less common forms of meningitis. Infections due to intestinal organisms, commonly coliform bacteria, most frequently met with in newly born babies, have been treated with some success by Dehre, Mozziconacci, and Berkman (1954). These authors injected streptomycin at the first lumbar puncture if the cerebrospinal fluid looked turbid and then gave chloramphenicol by mouth. With this treatment 12 out of 24 babies survived.

When the responsible organism was *Klebs pneumoniae* (*Bact friedländeri*), streptomycin intramuscularly and intrathecally was successful in 1 case after ineffectual attempts to treat the patient with penicillin and chlortetracycline had been abandoned (Thompson, Williams, Williams, and Anderson, 1952). However, 1 case with this infection recovered after the administration of sulphadimidine alone in doses of 2 G 4 to 6 hourly (Montuschi, 1954).

Listeria meningitis. *Listeria* have been found occasionally in cases of meningitis. There is bacteriological evidence (Williams and Hornung, 1954) that one organism isolated from an infection of the central nervous system was sensitive to oxytetracycline and less so to streptomycin and chlortetracycline, another was sensitive to penicillin, bacitracin, erythromycin, chloramphenicol, and the tetracyclines. Of 8 strains isolated from different cases of meningitis at the Communicable Disease Unit of Los Angeles County Hospital, and tested for sensitivity to 7 antibiotics, 6 (not necessarily the same) were sensitive *in vitro* to penicillin, to streptomycin, and to chlor and oxytetracycline, and similar proportions of a smaller number to tetracycline and erythromycin (Dedrick, 1957). Chloramphenicol, however, inhibited 3 only out of the 8 strains. All the 4 patients who recovered were treated with penicillin and, in addition, streptomycin or chloramphenicol with one of the tetracyclines or a sulphonamide.

Leptospiral meningitis. There is no evidence that antibiotics have any effect on meningitis due to leptospira (Broom, 1953).

Brain abscess

Antibiotics have undoubtedly influenced the prognosis in brain abscess, but although these drugs sterilize the abscess cavity when appropriate measures are used, extirpation of the abscess walls is necessary for cure. Since the introduction of penicillin and other antibiotics a recovery rate of 50 to 60 per cent has been reported (Davis 1951). Ballantine and White (1953) describe the mortality at Massachusetts General Hospital, Boston, during 2 succeeding 5 year periods as 25 out of 31 cases between 1936 and 1940 and 10 out of 29 cases between 1946 and 1950 when the use of antibiotics had been established. None of the deaths in the later period could be ascribed to the spread of the infection. Moreover, none of the abscesses had its origin

in infection of the lung or pleural space indicating that treatment of the primary condition in these cases had been adequate. Whether the abscess is treated by repeated aspiration and instillation of a suitable antibiotic until such time as it can be removed with little risk, or opened and drained, depends for success on the accessibility of the lesion and the skill of the attending surgeon. With suitable antibiotics the former treatment is preferable, for little of the antibiotic penetrates the brain tissue and the well tried method of aspiration and instillation is the most suitable way of keeping the drug in contact with the infected abscess walls. When the condition of the patient permitted it, Ballantine and White (1953) were prepared to wait 24 to 48 hours in order to identify the infecting organism and determine its susceptibility before injecting any antibiotic. It is of interest to note that although penicillin and streptomycin are most frequently used, 2 other drugs which are employed systemically only with circumspection, namely bacitracin and neomycin, cause little or no toxic effects when instilled into a brain abscess. Doses of up to 10,000 Units of bacitracin, 5,000 Units of neomycin, 20,000 Units of penicillin, or 5 mg. of streptomycin, have been injected into the abscess cavity and into the intrathecal space without causing damage. If polymyxin is indicated, 0.5 mg. can be introduced into the abscess cavity and not more than 1 G. intrathecally (Leca and Cabrieses, 1955). With these bacterial drugs, all of which, except polymyxin, are readily soluble in water, most infections can be dealt with. Where the suitable antibiotic is not toxic in therapeutic concentrations, systemic administration is advisable to prevent metastatic spread.

Subdural empyema

It is regrettable to find that of 4 patients with subdural empyema secondary to frontal sinusitis, 3 died although the primary condition had been treated with antibiotics (Biehl, 1955). Bacteriological examinations were carried out on the pus from each of these cases and from these tests there was presumptive evidence that the antibiotics used should have been suitable. In one case only a few Gram positive cocci were isolated, in another *Staph. aureus* and non haemolytic streptococci, in the third β haemolytic streptococci and coforms, and in the fourth *Haemophilus influenzae*. The antibiotics given to the 3 patients who died were penicillin and/or streptomycin. Data about the susceptibility of these organisms to the antibiotics were not available. It is quite possible that the *Staph. aureus* was a penicillinase producer, and that streptomycin ceased to have any effect on the infections owing to their having become resistant during the preliminary therapy. In the 1 case which did recover, a battery of antibiotics was given: penicillin in doses of 600,000 Units 4 hourly, 1 G. of streptomycin 12 hourly, and tetracycline and erythromycin by intravenous injection post operatively. Had sensitivity tests been carried out from the time first diagnosis of frontal sinusitis was made, the correct antibiotic for each infection might have been administered.

Urinary Tract Infections

Most antibiotics are concentrated in the urine and the response of acute infections to treatment should on this account be prompt. That very small doses of the commonly known antibiotics were enough to clear the urine of

sensitive bacteria was shown by Gould, Bowie, and Cameron (1953). Unfortunately many infections follow some obstruction in the urinary tract. As soon as one infection has been cleared up, another appears in its place unless it is possible to ensure an unimpeded flow of urine. Another point to consider in dealing with urinary tract infections is the acidity or alkalinity of the medium in which the antibiotic has to act and the optimum pH for each antibiotic. Penicillin and chlortetracycline, for instance, are most efficient in an acid medium. Novobiocin also is an acid. Chloramphenicol and the other tetracyclines and vancomycin are amphoteric, their antibacterial activity being little affected by the pH, while streptomycin, neomycin, and erythromycin act best in an alkaline medium. Most organisms, when present in sufficient numbers, grow well in normally acid urine, although urine does exert a certain non specific inhibitory effect on some organisms (Gerstung, 1951). *B. proteus*, by splitting urea, makes the urine alkaline, and it is probably for this reason that streptomycin is effective without preliminary alkalization of the urine against some proteus infections in this situation. If the urine is not already alkaline, measures to make it so are needed when streptomycin, neomycin, or erythromycin is indicated. The constantly changing flora in the urine make it almost impossible to predict which should be the most effective antibiotic to use even when the invading bacterium is known. In a recent paper Lind and Swanton (1956) stated that between 1951 and 1955 an examination of 2,000 micro organisms showed that the percentage of common organisms invading the urinary tract which were susceptible to the tetracyclines and chloramphenicol had fallen each year. Whereas over 60 per cent of *Staph. aureus* isolated in 1951 were susceptible to penicillin, in 1955 less than 20 per cent were inhibited by this antibiotic. That permanent elimination of bacteria is rarely achieved, however, was noted by Lazarus and Wood (1952), who found that in all patients followed up for 6 months the original offending organisms reappeared within that time even when no clinical recurrence had taken place. None the less, 6 months after treatment with the commonly used antibiotics and *Gantrisin* these authors found that 559 of their 805 patients were clinically cured. They no longer showed cellular elements in the urine and had repeatedly negative cultures. The fall in the percentage of organisms susceptible to antibiotics has not, however, been uniform. For example, by the end of 1955, *E. coli*, the commonest bacterium associated with urinary tract infections, was still responsive in 60 per cent of cases. The response of enterococci was variable, between 20 and 30 per cent of these being inhibited by the tetracyclines or chloramphenicol. The percentage of *Staph. aureus* susceptible to penicillin had reached its lowest level in 1954 when little more than 10 per cent could be controlled and it ceased to be the antibiotic of choice in treatment. Less than 40 per cent of proteus species were inhibited by chloramphenicol, between 40 and 60 per cent of *A. aerogenes* were sensitive to the tetracyclines or chloramphenicol, most species being inhibited by the latter, and few if any species of *Ps. aeruginosa* were controlled by any of these antibiotics (Lind and Swanton, 1956). The position about this time was clarified by a carefully written article by Kass (1955). In Fig. 14 can be seen the relationship between the common urinary pathogens and their susceptibility to therapeutic concentrations of different antibiotics in the blood.

In another investigation covering over a thousand episodes of urinary infec

tion in St Bartholomew's Hospital, London, between late 1952 and 1953, Garrod, Shooter, and Curwen (1954) found that *Proteus morganii*, paracolon bacilli, and *Ps. pyocyanea* were generally resistant to the antibiotics penicillin, streptomycin, the tetracyclines, and chloramphenicol. However, this resistance had not become so prevalent as in the United States. Although the

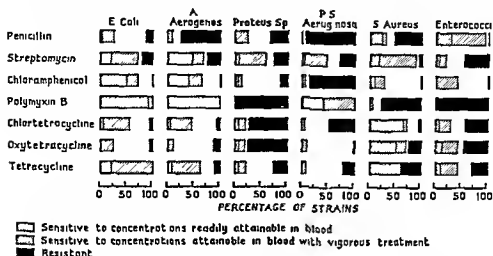


FIG 14 Sensitivity of common urinary pathogens to 7 antibiotics in relation to therapeutic concentrations in the blood. The gaps in the blocked-in areas represent percentages which are only inhibited by concentrations obtainable in the urine (After Kass *Amer J Med* 1955 18, 764)

Ordinary doses in G per day

| | |
|--------------------------------|---------|
| Penicillin (crystalline G I M) | 0.3-0.5 |
| Streptomycin | 1.0 |
| Chloramphenicol | 2.0 |
| Polymyxin B | 0.1 |
| Tetracyclines | 2.0 |

Doses for vigorous treatment

| | |
|-----------------|-----------------------------------|
| Penicillin | 50-500 |
| Streptomycin | 40 |
| Chloramphenicol | 40 or 10 I.V. in divided doses |
| Polymyxin B | 0.25 |
| Polymyxin E | 40 or 10 I.V. in divided doses |
| Tetracyclines | |

µg per ml which did not inhibit resistant strains

| | |
|-----------------|--------|
| Penicillin | > 1000 |
| Streptomycin | > 250 |
| Chloramphenicol | > 200 |
| Polymyxin | > 200 |
| Tetracyclines | > 200 |

majority of staphylococci were resistant to penicillin, they were generally still found to be inhibited by the tetracyclines and chloramphenicol, and no more than 17 per cent of enterococci were resistant to penicillin. How much further this increase in resistance has gone during the past 4 years remains questionable. Garrod *et al* (1954) also attempted to relate the sensitivity of organisms *in vitro* to the clinical result. When tests revealed that the causative bacterium was sensitive to the antibiotic used, the proportion cured reached 90 per cent in infections due to *Str. faecalis*, 82 per cent in those due

to staphylococci, 77 per cent in coliform infections, but only 60 per cent in those due to *Proteus vulgaris*. From a follow up study of patients treated successfully in hospital, these workers came to the conclusion that the greatest hindrance to successful treatment of an acute infection of the urinary tract with the appropriate antibiotic was either some abnormality or history of previous infection in the urinary tract. Where coliform bacilli were concerned Kirby, Corpron, and Tanner (1956) found that catheterization or instrumentation of the urinary tract were associated with antibiotic resistant strains. These patients would correspond to those with some abnormality in the urinary tract described by Garrod *et al* (1954). Coliforms which were sensitive were mainly isolated from patients who had acute symptoms on admission, a finding confirmed by Dutton and Ralston (1957). Polymyxin B held out most hope of retaining its activity against these bacteria, according to Kirby *et al* (1956).

Various workers have used combinations of drugs in the treatment of urinary infections. Spicer in 1950 demonstrated the favourable effect of using hacitracin together with polymyxin. Unfortunately both antibiotics have a nephrotoxic action and they should be a last rather than a first choice. Kaipainen (1952) showed experimentally that streptomycin with chloramphenicol or tetracycline could control *E. coli* with little sign of resistance to the antibiotics developing in repeated subcultures. This was later confirmed by Stern and Elek (1955) in about half the strains of *E. coli* which they tested. Without streptomycin only a minority of the organisms were actually killed by combinations of tetracycline and chloramphenicol although their growth was suppressed. Clapper, Sun and Meade (1957) observed that the addition of nitrofurantoin to a medium gave evidence of synergism between this agent and chloramphenicol or one of the tetracyclines against organisms isolated from infections of the urinary tract. No combination, however, was active against more than a few strains of pseudomonas species. In the clinical field, Pomerantz (1952) administered streptomycin together with chlortetracycline in a patient with a urinary tract infection due to *Ps. aeruginosa* and found that recovery was prompt. Other treatments, including polymyxin B, had previously failed. A more extensive trial was made by Rhoads, Billings, and O'Connor (1952) of a combination of an antibiotic with a sulphonamide, of mixtures of tetracyclines with chloramphenicol, and of streptomycin with penicillin sometimes with favourable results. Although slightly over 50 per cent of the 325 cases in this series were rendered free of bacteria during treatment, in a follow up period of several months only 8 to 15 per cent remained cured. (These were the patients without obstruction.) Other promising results from a combination of tetracyclines with chloramphenicol were described by Eisenberg, Alexander, and Flippin (1953).

Mention should be made here of neomycin, which is active against many Gram negative rods found in the urine (Waishren and Spink, 1950 *a* and *b*) as well as against the staphylococcus. This drug is bactericidal and does not readily induce resistance in the organisms exposed to it. A case of pyelo-ureterocystitis cystica due to *A. aerogenes* was described by Cox, Soanes, and Lowry (1955) in which, after many trials with other drugs, the patient experienced almost immediate relief when neomycin and the 2 streptomycins were administered together. Cultures of the urine became sterile within 48 hours and although from time to time symptoms and the aerobacter returned,

a single dose of 0.25 G of neomycin was sufficient to relieve the patient and to rid the urine of the invading organisms. Of the newer antibiotics little can be said at the present moment. Clinical trials of these drugs in urinary tract infections have shown cycloserine in conjunction with another antibiotic to be useful (Herrold, Bornd, and Kamp, 1955), and novobiocin in certain proteus and staphylococcal infections or those due to *Str. faecalis* (Trafton and Lind 1957 a).

Infections of the lower urinary tract

Prostatitis, in contradistinction to pyelonephritis, has been notoriously difficult to treat successfully even for a temporary period. It is of interest, therefore, to note how far the various antibiotics penetrate the tissues of the organs of the lower urinary tract as indicated by their presence in the secretions. Borski, Pulaski, Kimbrough, and Fusillo (1954) found little or no antibiotic in the prostatic tissue, fluid, or semen after the intravenous administration of penicillin, bacitracin, streptomycin, or chloramphenicol in amounts sufficient to produce therapeutic concentrations in the blood. A trace only was found in prostatic tissue after the administration of chlortetracycline, but a higher concentration was detected after the administration of oxytetracycline and tetracycline. Erythromycin was also consistently found in the prostatic tissue and semen when the average blood level was $1 \mu\text{g}$ per ml. When the infection in this region is susceptible to these drugs, therefore, the last 3 antibiotics are preferable to others. This is probably the reason why various workers have found oxytetracycline the most useful of antibiotics for infections of the lower urinary tract, for example non specific urethritis (Ambrose and Taylor, 1953; Doyle, Gill, and Lard, 1957; Harkness 1953; Parrino, 1954, and Willcox, 1957 a). A clinical trial of erythromycin was made by Borski *et al* (1954). All signs and symptoms disappeared in 9 of the 11 acute cases of prostatitis and urethritis or epididymitis, and considerable improvement was noted in 20 of the 34 cases with chronic infection. In view of the bacteriological results, however, where, except for a case with *Staph. aureus* infection, a considerable number of different organisms appeared during treatment, it is doubtful whether the therapeutic effect of erythromycin in lower urinary tract infections will be permanent.

In genito urinary wards, where the opportunities for cross infection are supreme, no routine therapy is therefore advised. If, on investigation, the prevailing bacteria in a ward are all found to be resistant to the common antibiotics, it would be wise to discontinue the use of all antibiotics for a certain period, for example 6 months, during which time every precaution should be taken to eliminate the spread of infection from one case to another. It is during this time that the no touch technique recommended by the Medical Research Council Memorandum (1951) should be introduced. It is not impossible even to pass a catheter without bringing it into contact with the hands. With care and skill it can be introduced by means of sterile forceps. Where it seems impossible to avoid contact of instruments or open lesions with the hands, dry sterilized gloves or mittens should be worn and changed with each dressing. Such precautions enabled Pyrah, Goldie, Parsons, and Raper (1955) to control an outbreak of *Ps. pyocyanea* infection, which at one time involved every patient in a ward of 17, and to reduce the incidence of this infection to a single case in over 4 months. Once infection within the

ward has ceased to spread, antibiotics can again be introduced with caution but only when suitable laboratory tests have indicated their use. When there is a choice, bactericidal antibiotics should be used in preference to those that are only bacteriostatic at therapeutically possible concentrations (for example, the tetracyclines). A routine pre-operative preparation should on no account be adopted. The continuous use of one antibiotic or combination of antibiotics in a unit is the ideal way to encourage the spread of resistant organisms. When these invade the patients' tissues the long post-operative convalescence is a much greater handicap in the quick turnover of patients than the preliminary day or two given to investigating the bacteriological aspects of a case thoroughly.

Infections of Bones, Joints, and Wounds

Acute haematogenous osteomyelitis

The great majority of these infections are still due to the staphylococcus but in many cases the organism is a penicillinase producer. In one of the more recent series described (Altmeier and Largent, 1952), when the prevalence of penicillin resistant staphylococci was near its maximum, penicillin was administered to all of 110 patients, and only 9 of these were found later to be infected with strains resistant to the drug. In these cases one of the tetracyclines or chloramphenicol was then used. As most of the patients were children Altmeier and Largent administered 50,000 Units of crystalline penicillin 3 hourly. This is advisable for the first 24 hours, after this time, if progress has been made, a long acting preparation can be used.

In acute infections, organisms other than the staphylococcus may be responsible, for example the haemolytic and non haemolytic streptococci, α haemolytic streptococci, pneumococci, other Gram positive cocci, *H. influenzae* type B, *A. aerogenes* (Altmeier and Largent, 1952), *E. coli* (Boyes, Bremner, and Neligan, 1957), or brucella (Löffler and Moroni, 1951). When seeing the patient in the early stages of the acute infection, however, the medical attendant will not know what the causal organism is, and if the child's condition is serious it is legitimate to start with penicillin. Only if the patient shows no response in the first 24 hours and the organism obtained by marrow puncture is found to be susceptible to penicillin *in vitro* are the tetracyclines or chloramphenicol to be used, a dose of 500 mg per kg body weight per 24 hours divided into 4 or 6 hourly doses is probably more than enough. If little response is observed after 24 hours the question of surgery will have to be considered in order to release the tension of the pus within the bone or under the periosteum. Surgery should be minimal in extent and the wound made by incision over the inflamed area should be closed at the end of the operation to prevent any secondary and susceptible invaders from gaining access to the inflamed parts. Treatment with penicillin should be continued for 3 weeks irrespective of the apparent quiescence of the lesion, for living staphylococci have been isolated after this time from some foci. Using penicillin in this way, or the other antibiotics mentioned above when the infection proved susceptible, Altmeier and Largent (1952) obtained excellent results with full healing and return of function in 50 of their 110

cases, good results, implying some limitation of movement, were obtained in 43, questionable effects in 14 and failures in 3, 2 of whom died. According to Bailey (1952) the effect of antibiotics has been to remove the picture of chronic osteomyelitis, carcinoma in a sinus tract, amyloid disease, and even the necessity for amputation.

In the newly born, where the source of infection is likely to be in the hospital nursery, *Staph aureus* still predominates but a majority of these organisms are resistant to penicillin (Boyes *et al*, 1957). In view of this finding these workers made a practice of administering a second antibiotic with penicillin—a tetracycline or streptomycin—at the time of their report. There are, however, now a number of antibiotics which should overcome the staphylococcus, one's choice being governed by the convenience of administration, the frequency of side-effects involved, and the liability of cross resistance to any other antibiotic in common use.

Chronic osteitis

When the infection has become chronic in bone a less dramatic effect is to be expected from antibiotics. There is moreover much less certainty about which are the organisms at fault. Bacteriological examination is, therefore, a necessary preliminary to giving antibiotics. When the bacteria present are known, antibiotics can be chosen according to their powers of inhibiting the organism concerned. It is possible that the bacteria isolated from a sinus mouth represent a superimposed infection rather than the organisms responsible for the primary infection in the bone. It is therefore advisable to operate when sinuses persist after a period of 10 days to 2 weeks of antibiotic treatment, as sequestra or sloughs are almost certainly responsible for the continuation of suppuration. When the focus is laid bare cultures should again be made of any dead tissue removed or swabs taken when no dead tissue is found. Staphylococci may then be found which did not appear at all or only as members of a mixed infection when cultures were made of the sinus mouths. Staphylococci are not the only organisms responsible for persistent inflammation of bone, various other infections may account for the condition. Cases of syphilis, actinomycosis, or blastomycosis were found in the series treated by Altmeier and Largent (1952). Fortunately penicillin could deal with the first two of these conditions, but amputation was the only method of arresting the spread of the blastomycosis. Although 2 patients with this condition recovered and 2 improved, a 5th case died from dissemination of the disease despite amputation. Venable and Pulaski (1950) considered that the following procedures should be carried out before operation in a focus of chronic osteitis was attempted:

- Eradication of bacteria laden tissue
- Provision of adequate blood supply
- Reinforcement of bone if necessary
- Full thickness skin cover

These authors also mention the need to compensate for the hypoproteinaemia which accompanies chronic suppurative disease. Frequent blood and plasma transfusions are advised until the haematocrit level shows a sustained reading of 50.

Acute purulent arthritis

Penicillin was again found to be an effective agent for the majority of 70 cases treated by Altmeier and Largen (1952). Chloramphenicol or one of the tetracyclines was used occasionally as indicated. Besides treating their patients by intramuscular injection, these workers also instilled 50,000 to 100,000 Units of penicillin into the joint cavity after aspiration, thereby providing a modified form of drainage as well as applying the antibiotic in its highest concentration at the site of the lesion. Treatment was kept up for 7 to 14 days. Even so, antibiotic therapy was not always successful and drainage had to be resorted to on occasion, particularly if the infection was mixed. Needless to say, treatment should be begun at the earliest possible moment. Irreparable damage has been done to the joints of babies where antibiotic administration was delayed (de Wet, 1954).

Compound fractures

Key (1951) described a programme for the treatment of compound fractures. This programme included the use of antibiotics. It is outlined here.

At the site of the accident. Antibiotics are administered only if it is known that it will be a long time before the patient receives medical attention in hospital. At least 100,000 Units, and preferably 400,000 Units of procaine penicillin G, are then given. A sedative, for example, morphine, to relieve pain and shock is administered. Tetanus antitoxin in a dose of 3,000 Units is injected or 1 ml. of the tetanus toxoid if the patient is already immunized.

On admission to hospital. A radiograph is taken only after some supporting splint has been applied. Operation is performed as soon as possible. A blood or plasma transfusion is set up before or during operation. After anaesthesia the dressings are removed, the skin cleansed with antiseptic soap and conservative excision of damaged tissue performed. A swab for culture is taken from the depth of the wound or from a piece of the dead tissue. Skin edges are excised only when crushed or badly contaminated. Necrotic material and foreign bodies are removed. The wound is enlarged and crushed muscle, if it does not bleed or retract when cut, is also removed. If a joint capsule and synovial membrane are involved the torn edges of these are excised and the edges of the membrane then gradually brought together. Tendons and nerves, if torn, are excised and repaired, and the bony ends, if grossly contaminated, are also excised. The fracture is reduced and fixed in place by internal or external fixation. Finally the wound is irrigated and if on inspection it appears good, without bleeding vessels or any foreign bodies, it is closed, provided that it has been operated on within 24 hours of the accident. If closure involves much tension this is relieved by lateral incisions, and if a flap is swung over to cover the wound, this should have a wide base and not be under tension. If there is much dead space left, drainage for 24 to 48 hours is advised. During all this time antibiotics are given: chlortetracycline, chloramphenicol or streptomycin and penicillin, according to the flora seen in Gram stained smears. The antibiotics are continued for a week to 10 days according to the appearance of the wound and the patient's temperature chart. In spite of antibiotics, when much laceration has occurred the surgeon must be continually on the look out for signs of clostridial infection. If any such signs occur, attention should be given to the blood supply

to the wounded area and a block of the lumbar sympathetic chain with procaine hydrochloride should be performed. The stitches should be released and the wound searched for signs of dead muscle. If this is found it should be removed. Clostridial infection was described by Prevo (1951) in a child of 2½ years who sustained a compound fracture of the ulna. Eighteen hours after prophylactic anti gas gangrene and anti tetanic serum had been given, and after reduction of the fracture and application of a plaster of Paris splint had been carried out, the child's hand was seen to be dark and the fingers were immobile. On removal of the plaster of Paris, a line of demarcation could be seen between the deeply discoloured skin around and above the laceration, and the normal skin at the junction between the upper and middle third of the forearm. Crepitation in the subcutaneous tissue, a distinctive odour, and radiological signs of gas beneath the deep fascia were also found. *Cl. welchii*, non haemolytic streptococci, and colon bacilli were cultured from the wound. A guillotine amputation was performed and 100,000 Units of penicillin were injected 3 hourly, together with 0.25 G. of streptomycin by intramuscular injection and 0.25 G. of chlortetracycline by mouth 4 times a day. An almost immediate improvement was seen in the child's general condition. The temperature and pulse became normal within 48 hours of operation and remained so, and the wound was fully healed 3 weeks from operation.

Infected wounds

Infection in clean post operative wounds

Attention was drawn to the rising incidence in post operative wound infections at the Massachusetts Memorial Hospital by Howe (1954). He traced this yearly from 1949 to 1953, and these were the figures he found following clean operations over a period during which there was widespread use of antibiotic prophylaxis.

| Year | No. of operations | Percentage becoming infected |
|------|-------------------|------------------------------|
| 1949 | 1197 | 1.09 |
| 1950 | 1129 | 1.77 |
| 1951 | 1209 | 1.98 |
| 1952 | 1193 | 2.51 |
| 1953 | 1229 | 3.98 |

In the great majority of these wounds *Staph. aureus* resistant to penicillin was cultured. Fig. 15 is a representation of the incidence of these post operative infections according to Howe (1957). Following a progressive yearly rise till the end of 1953, attention to the prevention of cross infection produced a temporary fall: the upward curve did not continue during 1957—personal communication from C. Howe. Adams (1957) also observed that in the 4 years preceding his report the infection rate in his hospital following 1,740 operations had been in the region of 0.4 per cent, but, in 1955, 8 infections had followed 603 operations—7 in 1 week in November. All these strains were penicillin resistant also.

In England, Blowers, Mason, Wallace, and Walton (1955) drew attention to a serious rise in the incidence of pyogenic wound infections in a thoracic surgery unit in 1952. Before 1949 the incidence had been between 1 and 2 per cent, but in 1950, 1951 and January to May of 1953 it reached the figures of 3.7, 2.1, and 10.9 per cent respectively. In 1952 *Staph. aureus* was found

in almost every clinically infected wound and the great majority were resistant to penicillin, though not, at this time, to chlortetracycline or chloramphenicol. Again, Shooter, Taylor, Ellis, and Paterson Ross (1956) followed the rise of post operative wound suppuration following 'clean' operations in a surgical unit at St Bartholomew's Hospital, London. During the period July 1954 to February 1955 the incidence was 9 per cent in 427 wounds. These wounds contained *Staph. pyogenes* usually resistant to penicillin. Clarke (1957) studied the infection rate in two general surgical wards over 10 months in wounds closed at operation. There were 382 of these and 52 broke down, or 13.6 per cent. In his series the commonest cause was the bursting of deep abdominal abscesses which on culture yielded coliform bacilli, but next to these the staphylococcus was the commonest cause, half of the strains being resistant to penicillin. These resistant staphylococci caused lesions as severe

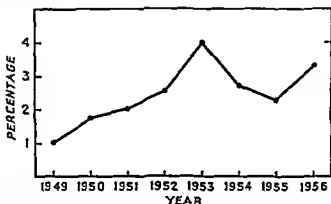


Fig. 1a. Infection rate for both major (serious) and minor (trivial) infections following clean operations over 8 years in the general surgical service of the Massachusetts Memorial Hospital. A progressive significant rise from 1949 to the end of 1953 despite routine use of prophylactic antibiotics during this time is seen. A fall in 1954 and 1955 followed an intensive programme for prevention of cross infection but despite these efforts the rate went up again in 1956.* (From Howe, *Ann Surg* 1957 146, 394.)

* More recent studies to the end of 1957 showed that the upward trend in 1956 was not maintained (C. W. Howe—personal communication).

as those caused by penicillin sensitive staphylococci. In 1956 (Shooter, Griffiths, Cook, and Williams, 1957) another outbreak occurred in a second unit where sepsis developed in 24 wounds following 86 clean operations, and *Staph. pyogenes* was recovered from 17 wounds. At this time all strains were resistant to penicillin, but though at first sensitive to chloramphenicol and erythromycin, as these antibiotics were introduced for therapy, the strains were found to be resistant to them as well. In addition to various methods to control the spread of infection, such as control of direction of currents of air, more extensive sterilization of instruments and materials, meticulous wound management with rigid aseptic methods, and isolation of carriers of epidemic strains of staphylococci, the simple measure of abandoning the routine use of antibiotics as a prophylactic measure for clean operations and reserving their use for established infections only was adopted by the Massachusetts Memorial Hospital. The 3 years following this were marked by falls in the incidence of post operative infections to the regions of between 1 and 2 per cent of operations (Keefer, 1957). The removal of antibiotics from the

field of pre operative prophylaxis means that reliance is placed on the resources of the surgeon and his staff to ensure the preventive measures needed to keep out bacterial invasion of operative wounds. Should this vigilance be relaxed there is little doubt that the incidence of infection would rise. Although having no influence on reducing the incidence of infection, there is, however, the possibility that the diminished use of antibiotics may lead to their again being active in controlling sepsis. An increase in infection rate was observed by Howo (1957) in the year 1956. It remains to be seen whether this was controlled by enforcement of the measures already outlined.

Battle casualties

Wounds received in battle, where there is much laceration and presence of foreign bodies, depend more on vigorous early surgical treatment than do those in civilian life. During the first 2 months of the Korean war, when casualties of the Commonwealth Forces had to undergo a long evacuation with many transfers until they reached their base hospital in Japan 42 hours to 6 days after boarding ship in Korea, there was ample opportunity for wounds to become septic or for gas gangrene to develop. In describing the condition in 1,850 patients received on board the hospital ship *Maine*, Latta (1951) stated that further surgical treatment was required in 12 per cent of 410 major wounds, including 180 compound fractures, 2 deaths followed in 82 cases of intrathoracic wounds involving all combinations of pneumo and haemo thorax, 3 deaths among 40 abdominal injuries and 2 each among 19 head injuries, and 19 burns. Although the fear of manifest infection led to further surgical toilet in patients where 'local pain, toxæmia, haemorrhage, unhealthy smell of the dressing or evidence of gangrene were present', yet Latta mentioned 3 cases of gas gangrene only and 3 cases of large abscesses amongst his many casualties. Procaine penicillin, 300,000 Units, was injected twice a day into all patients who had other than minor wounds, with the addition sometimes of streptomycin and chlortetracycline. How far antibiotic therapy was responsible for this low incidence of obvious infection cannot be gauged, but it is unlikely that surgical technique superior to that practised in the Second World War was responsible for the low mortality, or that restorative transfusions were practised to a greater extent. Again, when the evacuation of casualties had become better organized in the third phase of the war, Wright (1956) still considered that the long line of evacuation put a great strain on the men's endurance. One third of them took 6 or more days before secondary surgery could be performed in the British Commonwealth General Hospital in Kure, Japan. Most patients, in addition to having had their wounds debrided in the mobile army surgical hospital in Korea, had received penicillin and anti tetanus serum, yet not more than about 9 per cent arriving in Kure were found to be infected, 2 per cent severely so. No gas gangrene was seen. It is of interest to note that infections with *Ps. pyocyanea* or *Proteus* species were the most difficult to eradicate. When the organisms were sensitive *in vitro* to streptomycin or one of the tetracyclines the infection usually yielded to these antimicrobials, but correlation between *in vitro* tests and the results of chloramphenicol therapy was unsatisfactory.

Otolaryngological Infections

The choice of treatment for acute infections of the upper respiratory passages has already been dealt with and the conclusion reached that, unless the infection appears to be due to *Str pyogenes*, it is better treated with antipyretics and sedatives, as the chances are that a virus is responsible for the condition. When the discharge becomes purulent, however, or the tissues of larynx and pharynx give the appearance of a cellulitis, antibiotics are indicated. Bischoff (1955) claimed that the dust laden winds prevalent in western Texas were apt to be responsible for acute bacterial infection in the sinuses or pharynges, especially in children. During a 5 year study the majority of cases responded to 300,000 Units of procaine penicillin given intramuscularly or to oxytetracycline taken by mouth. When fever was present a vasoconstrictor was used, followed by one of the various somewhat toxic antibiotics as local insufflations. Bacitracin, neomycin, or polymyxin B, together with hyaluronidase, were applied with the result that fever subsided and prostration was alleviated within 24 hours.

Sinusitis

Davison (1951) made yearly tests of the sensitivity of the Gram positive cocci found in pus from the noses of patients with chronic sinusitis, and found a steadily decreasing proportion which remained sensitive to 0.02 Unit per ml of penicillin. Thus, between 1943 and 1950 the percentage of sensitive organisms had fallen from the region of 80 to zero. At this time Davison found that the greatest number of organisms were susceptible to chlortetracycline and oxytetracycline in concentrations of 1 to 5 μg per ml. Nevertheless, in acute cases fortified procaine penicillin in a dose of 4.8 million Units daily still produced a good response in the majority of cases, and with the addition of 0.5 G of streptomycin 6 hourly some of the best results were obtained. In chronic sinusitis 6.4 million Units of fortified procaine penicillin cleared up the condition after lower doses had failed.

Frontal sinusitis

If a comparison can be made between the morbidity of a condition treated in hospitals in different countries, the figures of Gill Carey (1954) are of particular interest. For 15 years up to 1944 the number of patients with frontal sinusitis admitted to the Massachusetts Eye and Ear Infirmary was 276 approximately half of these were treated before chemotherapy or antibiotics had been introduced. Four hundred and forty seven operations were performed on these patients, of which 34.4 per cent failed to achieve their objective. The mortality was 15 per cent, all but 1 death occurring before chemotherapy. In the 6 years preceding January 1954, 132 patients with the same condition were received at the Royal National Ear, Nose and Throat Hospital, London, when chemotherapy and antibiotics were in use and little change had been made in operative techniques. There were 29.5 per cent of operative failures, but only 1 patient died. This very great improvement on the earlier figures coincided with the introduction of the new agents and provides strong circumstantial evidence that they were mainly responsible for the improved prognosis.

Phlegmonous laryngitis

When the larynx was the site of acute cellulitis, chlortetracycline was administered in doses of 0.25 G 6 hourly by Rosenbaum (1954). In this worker's experience improvement followed in 5 out of 8 cases within 24 hours. It is obvious that in acute pyogenic infections, when no delay can be allowed for bacteriological tests before treatment is applied, the choice at present lies between penicillin, chloramphenicol and the tetracyclines. Whichever antibiotic is chosen should depend largely on the locality in which the patient contracted his illness. If at home, penicillin is still likely to be of benefit, if in a hospital where antibiotic resistant staphylococci are known to be prevalent, one of the tetracyclines should be chosen. On the other hand, if cases here have been found in general not to be responding to these drugs, then one of the newer antibiotics should be administered—erythromycin, novobiocin, or vancomycin.

Nasal conditions

Apart from the common cold, infections of the nose are not common. Those which supervene on an atrophic rhinitis, however, can be bacterial in origin. Sterstein (1951) dealt with 13 such cases, and later in 1954 with a further 22. Sterstein's initial treatment was to administer streptomycin and penicillin 4 times a day as an inhalant, together with a vasodilator and nicotinic acid. Ten of these early cases showed striking clinical improvement, with disappearance of odour, crusts, and *Klebsiella* organisms; there was a demonstrable increase in moisture on the surface of the nasal mucosa, an increase in circulation, and a partial return of the sense of smell. Later trials were made with other antibiotics: oxytetracycline, chloramphenicol, and bacitracin. Again striking improvement was seen in the majority of cases but there was still a small minority (2 in this series) which did not respond to treatment. It is possible that the underlying condition rather than the bacterial infection was responsible for the symptoms in these unresponsive cases.

External otitis

Otitis externa, although in the nature of an eczema, readily becomes infected. The most common organism to be isolated from the surface lesions is *Pseudomonas pyocyanea*, but *Staph. aureus*, *E. coli*, and *Staph. albus* are also found (David, 1951). Most workers agree that the results of local application of penicillin to the ears in this condition have been disappointing. Streptomycin followed by chloramphenicol cream has, however, been frequently useful. Oxytetracycline by mouth together with triple sulphonamides or *Marfanil* in a 1 to 4 per cent solution was advised by Tremble (1955). When *Ps. aeruginosa* was present in pure culture or when Gram positive cocci were absent, polymyxin B or E were particularly effective owing to their bactericidal action. A few cases are due to fungi, and these seem to be on the increase since no fewer than 29 were admitted to the Royal Victoria Hospital, Montreal, in a recent period of 30 months (Stuart and Blank, 1955). All were due to one strain or another of *Aspergillus*. Although local applications of neomycin, polymyxin, chloramphenicol, *Merthiolate*, or *Cresatin* were employed, the response was poor in all cases. It remains to be seen what effect the local or systemic administration of nystatin or some of the newer antifungal antibiotics may have on this condition.

In a comparative study of different treatments among 493 cases with acute diffuse otitis externa, Senturia, Cross, Lett, and Hardy (1954), using oxy-tetracycline, polymyxin B sulphate, 4 aminomethylbenzenesulphonamide hydrochloride, nitrofurazone, or oxytetracycline and polymyxin together, found that the relief of pain and tenderness, and the subsidence of oedema was generally achieved by oxytetracycline or oxytetracycline plus polymyxin B

Otitis media and mastoiditis

When occurring in babies this condition is often difficult to diagnose for the symptoms are often those of a gastro intestinal upset. It is important therefore always to look at the ear drums when examining a baby with a complaint the cause of which is not particularly obvious. At the time of reporting, Bourgeois and Franck (1951) found that chlortetracycline, in doses amounting to 0.5 G daily, led to rapid improvement in this condition. If the response did not follow quickly, chloramphenicol or oxytetracycline were tried. Penicillin and the sulphonamides were considered to be too often useless for trial before the drum had perforated and an opportunity of isolating the responsible organism and testing its sensitivity had presented itself. When the ear drum is bulging or there are signs of much exudate in the middle ear, myringotomy should be performed, for the retention of inflammatory products, even when the infection is controlled, leads to thickening of the membrane and lessening of the mobility of the ossicles, so that permanent impairment of the acuity of hearing may result (Flanders, 1950, Lederer, 1950, and McLaurin, 1953. Popper, 1957). In older patients, especially if they had received antibiotics, *Bact. coli* and *Ps. pyocyanea* are frequently found. When the ear is already discharging, inability to make a bacteriological examination is no longer a barrier to correct therapy. Careful cleansing of the ear with equal parts of penicillin and streptomycin usually cleared the discharge in 3 days (David, 1951). An interesting year's survey of the treatment of otitis media in general practice in this country was made by a working party of the Medical Research Council (1957 b). In 13 practices throughout the country in 1955, 1,162 patients were diagnosed as having acute otitis media, i.e. they complained of pain or deafness and on examination showed redness of the drum, discharge, or perforation. Treatment varied from practice to practice: in 7, more than 90 per cent. of patients received an antibiotic or a sulphonamide, whereas in 3 others chemotherapy was employed for less than 40 per cent. of the patients. Penicillin was used to the greatest extent but the tetracyclines, chloramphenicol, or streptomycin were also prescribed. Assuming that this method of treatment was only used when necessary, the results are of interest: in 1 patient only was myringotomy performed, but there were 14 who were referred to hospital about whose subsequent history nothing is known. In the remainder, complications occurred in 8 patients in the shape of mastoiditis, cervical adenitis, facial palsy, and meningitis 7 weeks after the attack of otitis media. Six months after the attack all but 50 patients had been followed for signs of residual defects. This occurred in 8 per cent. of patients, more commonly as age increased. Deafness, usually slight, was the most usual after effect and discharge was present in 17 patients.

Mastoidectomies are now seldom performed and the complications which follow otitis and mastoiditis were rare by 1951 (Davison). Cases requiring

mastoidectomy steadily diminished even when only penicillin was used. Davison (1951) quotes the following figures from the Geisinger Memorial Hospital, Pennsylvania

| Year | Drug in current use | No of cases with acute otitis and mastoiditis | No with simple mastoid operation | Percentage requiring mastoidectomy |
|------|-----------------------------|---|----------------------------------|------------------------------------|
| 1937 | | 203 | 119 | 59 |
| 1943 | Sulphonamides | 283 | 74 | 26 |
| 1946 | Penicillin (in small doses) | 247 | 10 | 21 |
| 1949 | Penicillin (in large doses) | 189 | 5 | 2.7 |
| 1950 | Penicillin | 93 | 5 | 5.3 |

(First 6 months)

The rise in the percentage of patients requiring mastoidectomy was ascribed to the greater prevalence of penicillin resistant organisms

Later publications have been more concerned with the results of antibiotic therapy than with the specific treatment indicated. Obviously with such a battery of drugs now readily available—penicillin, bacitracin, streptomycin, the tetracyclines, neomycin, polymyxin, erythromycin, novobiocin, and others—it should be difficult to find no drug which would control the infection under consideration. *Staphylococci* and *haemolytic streptococci* still seem to predominate in infections of the paranasal sinuses, pharynx, and surface of the eye (Prigal, Molomut, and Maher, 1951), and since local applications of a drug are often as efficacious as systemic administration, there is little to be feared from toxic effects. Reardon (1952) like Davison (1951), stated that mastoidectomy had almost disappeared from the surgeon's curriculum, that Caldwell Luc operations in the maxillary sinuses were uncommon, and that radical frontal operations were rarer still. Intracranial complications arising from infected sinuses had also decreased remarkably. Stinson in 1951 stated that in the previous 4 years he had had no case of mastoiditis in patients he had attended for the original otitis, all these patients had received antibiotics from the beginning of treatment. Rutter and Ballantyne (1952) had little success with sensitivity tests in the 54 cases with discharging ears and fell back for the most part on local insufflation of powdered oxytetracycline. With such disparity between clinical results and sensitivity tests as described by these authors, one is tempted to suggest that the method of testing for susceptibility should be looked into before this surest guide to the right treatment is discarded. Fowler and Freeman (1954), on the other hand, relied on sensitivity tests as a guide to treatment and found that 50 per cent of cases responded to the tetracyclines or chloramphenicol. These authors agreed with Rutter and Ballantyne (1952), however, that oxytetracycline powder was particularly successful, although polymyxin B was also recommended for application to certain infections of the ear. One other group of workers investigated the position in chronic otitis media (Das, Singh, Taneja, Khanna, and Chaddah, 1954). In cases of chronic otitis media the following antibiotics were found to be most suitable

For proteus and *Ps. pyocyanea* infections

Dihydrostreptomycin drops until the ear was dry, then the powdered antibiotic insufflated into the ear

| | |
|--|-----------------|
| For staphylococcal infections and those due to <i>Str viridans</i> | Oxytetracycline |
| For infections due to the coli aerogenes group | Chloramphenicol |

In view of the knowledge that the organisms causing these conditions could be found in the external auditory meatus for as long as 3 years after healing, insufflations were continued for 1 year after the ear had dried. Whether this was a wise precaution cannot be inferred from the results.

Infections of the Eyes

Before considering the various infections of the eye it is necessary to discuss the most suitable routes of administration for the different antibiotics in order to secure their penetration into the tissues and chambers of the eye. Sorshy (1950) has pointed out that the eye is pharmacologically a double organ. The lids, conjunctiva, adnexa, and sclerotic have a similar blood supply and can be grouped with other organs, the general rules for the administration of antibiotics applying to these tissues also. The cornea, the aqueous, and the vitreous are avascular and the concentrations attainable in these vary with the mode of administration. Subconjunctival injection undoubtedly provides the most effective method of administration, for it not only produces higher concentrations than are obtainable by local application or with the usual systemic doses, but the level is maintained for a relatively long period. If a vasoconstrictor such as adrenaline is added to the injection, a dose of 1 million Units of penicillin produces concentrations in the vitreous comparable with those obtained from direct injection of 5 000 Units into this humour, without the risk of trauma and consequent impairment of vision. Similar results are produced by streptomycin, but unfortunately chlortetracycline, oxytetracycline, and chloramphenicol are rather irritating. Chloramphenicol, however given systemically, rapidly penetrates the ocular tissues which have a good blood supply (Dunphy, 1950).

Blepharitis

According to Ainslie (1951), strains of staphylococci responsible for this condition were by 1951 already poorly sensitive or definitely resistant to the action of penicillin and sensitization had become more common. There were thus two reasons for using this antibiotic with restraint. In any case, before using it, a culture and a test of sensitivity should undoubtedly be made. When tests were made in 30 cases of blepharitis, only 11 out of 22 staphylococci isolated were sensitive to penicillin whereas 21 were inhibited by chlortetracycline as judged by the paper disk method of testing. The results of the clinical trial with chlortetracycline were not so encouraging, for, of the 22 cases only 13 were successfully treated, in 6 the infection recurred and 3 cases did not show any improvement. Thus only 2 more cases responded to treatment than might have been expected had penicillin been used. In a few cases, however, *Bact friedlander* was isolated, or no bacteria were cultivated from the lids. These cases responded well to chlortetracycline after the crusts had been removed with warm saline, an ointment containing 1 mg per ml was applied 2 hourly, and later 4 hourly.

Keratitis

Whether penicillin should be used in infections due to penicillinase producing staphylococci is still open to doubt. Locke (1949) used it in rabbits with anterior chambers infected experimentally with 3 separate strains of organism. One of these strains produced too mild an inflammation to be worth testing, but the inflammation produced by the other 2 strains was treated with solutions of sodium penicillin containing 1,000 Units per ml, bacitracin containing 1,000 Units per ml, or streptomycin or a combination of penicillin and streptomycin given by iontophoresis. Curiously enough, the infection caused by 1 strain responded best to penicillin, but the other strain was not affected. Neither infection benefited from the combination of penicillin and streptomycin. When bacitracin was used, no greater concentration than 1,000 Units per ml could be tolerated, for stronger solutions produced conjunctival congestion, chemosis, and blepharospasm.

Trachoma

In the acute and early stage trachoma can be readily brought under control by the sulphonamides. It is when the condition has become chronic and scarring is present that treatment becomes problematical. Bietti, who made an intense study of the effect of antibiotics on trachoma in 1948 when he had only penicillin, streptomycin, and tyrothricin for trials, found penicillin the only effective agent. Siniscal (1950) gave modified support to this conclusion. From observations on more than 3,000 cases at the Missouri Trachoma Hospital during 1941 to 1948 Siniscal considered that the sulphonamides had proved their efficacy as the agents of choice and that penicillin and bacitracin played their part only by clearing up the secondary infection. Freyche (1950) noted, as did Bietti, that under the influence of penicillin inclusion bodies degenerated and eventually disappeared. Unfortunately invasion of the conjunctival epithelium frequently recurred when treatment was stopped. Freyche considered that penicillin acted in a different way from streptomycin, the latter being useful only in controlling the secondary infection which so frequently accompanies trachoma. Chlorotetracycline, however, was at that time found to act at least as well as penicillin or the sulphonamides in the one case in which it was tried. From reports received from others (Moutinho, Grilo, and de Moura, 1949), however, Freyche stated that of 39 cases treated by chlorotetracycline at all stages of the infection 27 showed great improvement or were cured, corneal complications also responding rapidly to treatment. Chlorotetracycline, when locally applied, is apt to be irritating and for this reason it is better to choose one of the other tetracyclines. Both, applied locally and persistently several times a day in strengths of 0.5 to 1 per cent in ointment, have cleared up trachoma whether acute or chronic, the cicatricial stage being the only one beyond control. This treatment was advocated by the Expert Committee on Trachoma of the World Health Organization in 1952.

Isolation of 3 strains of the virus of trachoma was claimed by Tang, Chang, Huang, and Wang (1957). Curiously enough, they found that they were readily inactivated not only by the tetracyclines but by penicillin. They were, however, resistant to streptomycin. A later report of the same Organization (1955) pointed out that 400 million people, or one sixth of the world's

population, were infected with trachoma, only Europe and the North American continent being considered relatively free of the infection. Mass campaigns instituted in Egypt, Tunisia, Morocco, Formosa, and elsewhere proved the disease to be curable with a combination of antibiotics and sulphonamides. In view of the expense of the tetracyclines the treatment recommended by the World Health Organization was reduced to 2 daily applications of antibiotic ointment over 2 months together with treatment of the accompanying conjunctivitis. Three years' trial of this limited number of applications had proved satisfactory.

Infections of the Skin

In 1954 the Medical Research Council of Great Britain issued a report on the results of treatment with different antibiotics in the commoner dermatoses. This provides a good foundation for discussion and is therefore summarized here. Three antibiotics were chosen—chlortetracycline, oxytetracycline, and chloramphenicol, the fourth medication being a dummy capsule indistinguishable in appearance from the others. Each patient was given 4 doses daily amounting to 2 G together with a vitamin B tablet. The treatment was continued for 3 weeks. The results were as follows:

Herpes simplex recurrens (31 cases including 8 controls) no effect obtained, 1 case relapsed while under active treatment

Plantar warts (30 cases including 9 controls) no indication was given of the superiority of antibiotic therapy over administration of inert capsules

Lichen planus (19 cases including 4 controls)

Pityriasis rosea (25 cases including 6 controls)

Dermatitis herpetiformis (12 cases including 2 controls)

Discoid eczema (16 cases including 3 controls)

Seborrhoeic infective eczema (7 cases of short duration) these made a rapid recovery

(7 cases of long standing) 5 of these were unaffected

The results with antibiotic treatment were comparable to those of the controls

With these antibiotics nausea, vomiting and other gastro intestinal disturbances were severe enough to require discontinuance of treatment in 29 out of 147 patients. These conclusions indicate that the antibiotics used have a single function, that is, to control infection and that conditions of a different aetiology do not benefit from antibiotics except when the lesions become secondarily infected.

On the other hand there have been many reports of the good effect produced by antibiotics in pyogenic dermatoses. Philip (1954) used an application containing 500 Units of bacitracin and 10,000 Units of polymyxin B sulphate added to 55 ml. of water and mixed in a base of carbowax, vegum, sorbital, and lecithin. The lotion was painted on the affected areas 3 times a day and allowed to dry so as to form a protective coating over the lesions, no bandaging was applied. These 36 cases, which included varicose and exudative eczema, contact dermatitis, chronic paronychia, hydradenitis suppurativa, *Rhus* poisoning, and dissecting cellulitis, were obviously infected. Excellent results were obtained in cases which were acute and weeping but, as might

be expected from the poor access of the drugs to the lesion resulting from local application, only a fair result followed treatment of dissecting cellulitis. Leg ulcers were promptly sterilized, permitting surgical procedures for covering them, and exudative dermatitis dried promptly, as did the bullous lesions of pemphigus, although the pemphigus itself was unaffected. Deeper coccal infections such as sycosis vulgaris and folliculitis responded so long as they were accessible from the skin surface, and secondary purulent infections cleared rapidly. In closed lesions where no penetration of the lotion was possible, there was no effect. From this report as well as others, for example, Miller, Slatkin, and Johnson (1948), it can be seen that when used locally for susceptible infections bacitracin is fully as effective as penicillin. Reactions up to the present time have been very few, so that there is little fear of these complicating the issue. Since neomycin is an all purpose drug and both neomycin and bacitracin are bactericidal, the combination of the 2 for infective dermatoses seems to be approaching the ideal. Always, however, one must add the proviso that routine and widespread use of the drugs may encourage the predominance of resistant strains.

Pyoderma Impetigo

Livingood (1951) made a special study of the treatment of this condition in children. Among the bacteria isolated from the lesions were *Staph aureus*, haemolytic and non haemolytic streptococci, *Staph albus*, *Str viridans*, *Alkaligenes faccalis*, *Staph citreus*, proteus, and *Ps pyocyanea*. Livingood found the most satisfactory treatment to be soaks in potassium permanganate, 1:9,000 to 1:16,000, and, if the usual antiseptic applications such as ammoniated mercury ointment, Burow's solution silver nitrate, Vioform, or Castellani's paint were not satisfactory, a suitable substitute was an ointment containing bacitracin in a concentration of 500 Units per G, chlortetracycline 30 mg per G, and neomycin 1.5 mg of the powdered sulphate to 1 ml of water. On the other hand, if the involvement of the skin was extensive, systemic therapy became mandatory, penicillin, chloramphenicol, or one of the tetracyclines, in this order of preference, was then given. Noojin (1951) advised treatment for pyoderma on much the same lines, but still recommended penicillin in preference to other antibiotics when systemic administration was advisable. However, for local application Noojin pointed out that tyrothricin was still available and was stable as a wet dressing so that it could replace penicillin or, if need be, bacitracin. Again the reader is reminded that where *Ps pyocyanea* predominates polymyxin is the most reliable of the antibiotics for removing it.

Another series of pyogenic infections treated by Miller *et al* (1955) with erythromycin (10 mg per G in a petrolatum base), neomycin in the same concentration, or oxytetracycline (30 mg per G), showed that each antibiotic alone produced results which were as satisfactory as when two of the antibiotics were used together. Impetigo, folliculitis, eczematoid dermatitis and other infected dermatoses all responded better in patients treated with erythromycin or oxytetracycline than in those treated with a placebo.

At the present time administration of combinations of novobiocin with tetracycline phosphate complex (James, 1957), or tetracycline with nystatin (Rein, Lewis, and Dick, 1957) seems to be the favourite method of treating pustular dermatoses. Therapeutic responses in a variety of conditions

including furunculosis, pyoderma, impetigo, sycosis vulgaris or barbae, infectious eczematoid dermatitis, hidradenitis suppurativa, dermatitis repens, and acne vulgaris were excellent in the great majority of cases. But the evidence in this book shows repeatedly that the antibiotic which produces an excellent response today, if used routinely, will tomorrow be of little value.

The addition of cortisone to the various antibiotic preparations was tried by Robinson, Robinson, and Strahan (1955). These authors found that cortisone produced a temporary involution of lesions and a relief of symptoms, but when it was used alone relapses occurred, these did not occur when antibiotics were used at the same time. These authors used 10 preparations consisting of one or other of the tetracyclines, erythromycin, neomycin, neomycin and bacitracin, or these two together with polymyxin, but no preference was stated.

Dermatoses due to yeasts

From experiments *in vitro*, Graciansky, Leclercq, Delaporte, and Gouin de Roumilly (1955) saw no evidence that antibacterial antibiotics stimulated the growth of yeasts or that these organisms acquired greater virulence when growing in the presence of an antibiotic. When, however, these workers turned their attention to animals they found that the administration of the antibiotics then in their possession did favour the growth of yeasts. They explained the phenomenon by an alteration in microbial pattern which allowed the yeasts to grow more vigorously when the antibiotics were administered.

The discovery of various antifungal antibiotics, of which nystatin has been most extensively developed up to date, has changed the picture. A limited clinical trial of local applications of nystatin ointment or solution 3 times daily to sites of cutaneous moniliasis, and of oral administration of the drug in 59 patients produced good to excellent results in all. The length of treatment usually required to prevent relapses was 2 to 4 weeks (Wright, Graham, Newcomer, and Sternberg, 1956). In a second study by Wright, Graham, and Sternberg (1957) nystatin was confined to local applications. Results were still good in the great majority of cases, though brought about more slowly. R. C. V. Robinson (1956) also observed consistent improvement in lesions about the nails and fingers after applications of nystatin in an ointment containing 100 000 Units per G.

Acne

This stubborn condition only sometimes responds to antibiotics. Robinson (1954) found only 19 of his 72 patients were relieved by the use of antibiotics. Penicillin administered intramuscularly was found to be of no value, and the tetracyclines, chloramphenicol, and erythromycin were only considered of value as supplementary methods of treatment. After an initial improvement has been obtained by these drugs they should not be continued, but used only intermittently in relatively small maintenance doses.

In pustular acne, Noojin, Osment, and Winkler (1954) found that the predominant organism was a haemolytic *Staph. albus* against which penicillin was the most effective antibiotic but oxytetracycline, chlortetracycline, and chloramphenicol, in that order of effectiveness, came next. Sensitivity tests

of the organisms isolated were made to determine which antibiotic was most likely to be effective. As a result of these tests, 17 of the 24 patients in this series received 100,000 Units of penicillin by mouth 3 times a day, 2 patients received chlortetracycline, and 3 oxytetracycline. The pustules improved within 2 weeks in 20 patients and further improvement was noted when the antibiotic was changed. No response was obtained in 3 cases. When, however, any of the antibiotics were discontinued relapse was apt to occur. A combination of oleandomycin with tetracycline applied 4 times a day for 8 weeks proved successful in 80 per cent. of cases in the hands of French and Stutzler (1957), a better result than that obtained with oleandomycin alone, neomycin, novobiocin, or combinations of these antibiotics with other tetracyclines. This combination, however, did not impress Cornbleet and Firestein (1957) as being particularly effective during a similar period of treatment. These observations seem to indicate the need for more prolonged antibiotic therapy and regular bathing of the site with some non irritating antiseptic solution, so that the organisms will not persist on the skin and re invade the tissues as soon as the protection afforded by the antibiotics is withdrawn.

Acne necrotica

Only 2 instances of this condition treated by antibiotics have been found (Stritzler, Friedman, and Loveman, 1951). Even though the infection had persisted for several years with ulcerated and crusted lesions along the margins of the scalp, forehead, nose, and eyebrows, it was controlled by penicillin in doses of 100,000 to 300,000 Units of the crystalline preparation daily, or the same dose of the oily preparation twice weekly. However, recurrence followed in 1 case after treatment was discontinued. The organism isolated during the recurrence required 8 Units of penicillin per ml. to inhibit growth, but no mention is made of whether or not the organism was a penicillinase producer, in which case a secondary infection might be suspected.

Hidradentitis suppurativa

From 23 cases treated at the Veterans Administration Hospital, Brooklyn, New York, by Steiner and Grayson (1955) a haemolytic *Staph aureus* was usually cultivated, but in 22 chronic cases various secondary invaders had taken the place of the staphylococcus. The acute form responded to simple measures such as the administration of a suitable antibiotic, incision and drainage, and the condition did not usually recur. In the chronic form, satisfactory results were more difficult to obtain. 'Intensive antibiotic therapy', irradiation and attention to the systemic disorders did not produce anything but poor results. Radical excision of the affected area with subsequent grafting seemed to be the only method which offered hope of success.

Ocular pemphigus

In the few cases seen by Church and Sneddon (1953) at the Royal Infirmary, Sheffield, antibiotics had no effect on the progression of the lesions.

CONCLUSION

The foregoing pages have accomplished little if they have not led the reader to conclude that the value of antibiotic therapy is in a continual state of flux. The changing susceptibility of pathogenic bacteria to antibiotics in common use within a restricted area, the commercial production of host factors which influence the bodily reaction to infection, the possibility of increasing numbers of antibiotics being prepared against disease even the chance that an agent may be found to destroy penicillinase, would alter the scope of the activity of antibiotics in a very short period of time. At the present time no clinician can claim to know which antibiotic he should prescribe, even when he knows the aetiological organism without being supplied at the same time with the results of a sensitivity test and without full knowledge of the characteristics of the drug which seems most suitable *in vitro*. Its liability to induce complications the most suitable route by which it should be given and the rapidity with which it may induce resistance in the infection to be overcome all need to be taken into account. If these precautions need to be taken with regard to a single antibiotic, still more do they apply to a combination of them. Lastly, routine use of antibiotics in hospitals, particularly as prophylactic measures should be abandoned and their use limited to those cases where they are specifically indicated.

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